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Midwest Research Inst, Kansas City, Mo.

Prepared for

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TOXICITY STUDIES OF SELECTED CHEMICALS

TASK II:

THE DEVELOPMENTAL TOXICITY OF VINYLIDENE CHLORIDE INHALED BY RATS AND MICE DURING GESTATION

FEBRUARY 1977

FINAL REPORT

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF TOXIC SUBSTANCES
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16. ABSTRACT
The purpose of this study was to determine the teratogenic potential of vinylidene chloride inhaled by rats and mice during organogenesis. This report describes the effects of vinylidene chloride (inhaled at various concentrations for different intervals during gestation) on development. Morphological and behavioral parameters were used to monitor development. Vinylidene chloride was judged to be a weak teratogen with little primary effect on reproduction.

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Final Report

Prepared by

Robert D. Short, Jr.
Jan L. Minor
Joseph M. Winston
Brett Ferguson
Timothy Unger
Cheng-Chun Lee

Contract No. 68-01-3242

Joseph Seifter
Project Officer

Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under Environmental Protection Agency Contract No. 68-01-3242, MRI Project No. 4128-B, "Toxicity Studies of Selected Chemicals." The work was supported by the Office of Toxic Substances of the Environmental Protection Agency. Dr. Joseph Seifter is the contract monitor for the project.

This work was conducted in the Biological Sciences Division under the direction Dr. William B. House between October 1, 1975 and November 1, 1976. The experimental work was supervised directly by Dr. Cheng-Chun Lee, Assistant Director, Biological Sciences Division for Pharmacology and Toxicology; assisted by Dr. Robert D. Short Jr., Mr. Jan L. Minor, Dr. Paul Peters, and Dr. Joseph M. Winston, Associate Toxicologists, with the technical assistance of Mr. Brett Ferguson, Mr. Timothy Unger and Miss Mary Sawyer.

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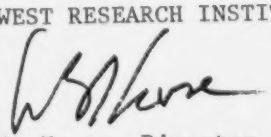

W. B. House, Director
Biological Sciences Division

TABLE OF CONTENTS

		Page
Summary		1
I.	Introduction	2
II.	Methods	3
	A. Experimental Design	3
	B. Exposure	4
	C. Animals	6
	D. Protocol for Morphological Evaluation	6
	E. Protocol for Behavioral Evaluation	7
	F. Statistics	9
III.	Results	9
	A. Chamber Concentrations of VDC	9
	B. Morphological Studies on Mice and Rats	11
	C. Behavioral Studies on Rats	40
IV.	Discussion	54
	A. Morphological Study	54
	B. Behavioral Study	59
	C. General Summary	60
References		61

LIST OF FIGURES

Figure	Title	Page
1	Diagram of Activity Maze	10
2	Cumulative Percent of Rats, Sexes Combined, Reaching a Criterion of 1 Sec or Less for Righting on a Flat Surface	47
3	Amount of Time Spent Pivoting During a 3-Min Test Period, Sexes Combined	48
4	First a Complete Startle Response Was Observed for Various Groups of Rats, Sexes Combined	49

TABLE OF CONTENTS (Continued)

LIST OF FIGURES (Concluded)

Figure	Title	Page
5	Cumulative Percent of Rats, Sexes Combined, Reaching a Criterion of 15 Sec on the Bar-Holding Apparatus.	50
6	First Day of Appearance for Righting in Air for Groups of Rats, Sexes Combined.	51
7	First Day for Score of 2, 3, or 4 in the Swimming Test, Sexes Combined.	52
8	First Day of Appearance of Physical Parameters for Groups of Rats, Sexes Combined	53
9	Average Body Weight of All Rat Pups	55
10	Continuous Activity of Groups of Three Rats (same sex) in a Residential Maze, Both Sexes Combined	56

LIST OF TABLES

Table	Title	Page
1	Chamber Concentrations of Vinylidene Chloride During Various Trials.	12
2	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 16 on Maternal Welfare and Reproduction in Mice. . .	13
3	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 16 on Maternal Welfare and Reproduction in Rats. . .	14
4	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 16 on the Incidence of Soft Tissue Anomalies in Mice.	16
5	Effect of Vinylidene Chloride on Gestational Days 6 to 16 on the Incidence of Soft Tissue Anomalies in Rats	17
6	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 16 on the Incidence of Skeletal Anomalies in Mice. .	18

TABLE OF CONTENTS (Continued)

LIST OF TABLES (Continued)

Table	Title	Page
7	Effect of Vinylidene Chloride on Gestational Days 6 to 16 on the Incidence of Skeletal Anomalies in Rats.	19
8	Effect of Vinylidene Chloride Exposure on Gestational Days 8 to 15 on Maternal Welfare and Reproduction in Mice. . .	20
9	Effect of Vinylidene Chloride Exposure at 41 ppm on Maternal Welfare and Reproduction in Mice.	22
10	Effect of Vinylidene Chloride (VDC) Exposure at 54 ppm on Maternal Welfare and Reproduction in Mice	23
11	Effect of Vinylidene Chloride Exposure on the Incidence of Gross Anomalies in Mice	24
12	Effect of Vinylidene Chloride (VDC) Exposure at 41 ppm on the Incidence of Soft Tissue Anomalies in Mice.	25
13	Effect of Vinylidene Chloride (VDC) Exposure at 54 ppm on the Incidence of Soft Tissue Anomalies in Mice.	26
14	Effect of Vinylidene Chloride (VDC) Exposure at 41 ppm on the Incidence of Skeletal Anomalies in Mice	28
15	Effect of Vinylidene Chloride (VDC) Exposure at 54 ppm on the Incidence of Skeletal Anomalies in Mice	29
16	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 9 on Maternal Welfare and Reproduction in Mice . . .	30
17	Effect of Vinylidene Chloride Exposure on Gestational Days 9 to 12 on Maternal Welfare and Reproduction in Mice. . .	31
18	Effect of Vinylidene Chloride Exposure on Gestational Days 12 to 15 on Maternal Welfare and Reproduction in Mice . .	33
19	Effect of Vinylidene Chloride Exposure on Gestational Days 15 to 17 on Maternal Welfare and Reproduction in Mice . .	34

TABLE OF CONTENTS (Concluded)

LIST OF TABLES (Concluded)

Table	Title	Page
20	Effect of Vinylidene Chloride (VDC) Exposure on the Incidence of Gross Anomalies in Mice.	35
21	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 9 on the Incidence of Soft Tissue Anomalies in Mice.	36
22	Effect of Vinylidene Chloride Exposure on Gestational Days 9 to 12 on the Incidence of Soft Tissue Anomalies in Mice.	37
23	Effect of Vinylidene Chloride Exposure on Gestational Days 12 to 15 on the Incidence of Soft Tissue Anomalies in Mice.	38
24	Effect of Vinylidene Chloride Exposure on Gestational Days 15 to 17 on the Incidence of Soft Tissue Anomalies in Mice.	39
25	Effect of Vinylidene Chloride Exposure on Gestational Days 6 to 9 on the Incidence of Skeletal Anomalies in Mice . .	41
26	Effect of Vinylidene Chloride Exposure on Gestational Days 9 to 12 on the Incidence of Skeletal Anomalies in Mice. .	42
27	Effect of Vinylidene Chloride Exposure on Gestational Days 12 to 15 on the Incidence of Skeletal Anomalies in Mice .	43
28	Effect of Vinylidene Chloride Exposure on Gestational Days 15 to 17 on the Incidence of Skeletal Anomalies in Mice .	44
29	Effect of Vinylidene Chloride Exposure on Gestational Days 8 to 20 on Maternal Welfare and Reproduction in Rats. . .	45

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

SUMMARY

Vinylidene chloride (VDC) was administered at various concentrations by inhalation to two experimental animal species for 23 hr/day (allowing 1 hr for servicing the animals and chambers) for various intervals during gestation. Charles River CD rats and CD-1 mice were used in these tests. The development of these animals was observed using morphological and behavioral parameters.

The results of this study indicate that (1) VDC is more toxic in adult mice than adult rats, (2) adverse effects on maternal welfare, as measured by weight gain, feed consumption, and survival were observed in both mice and rats, (3) although morphological changes were observed in fetuses from dams exposed to VDC, these effects were observed at concentrations that also affected maternal welfare, (4) no problems with neural development, as measured by behavioral parameters, were observed in rats exposed to VDC, and (5) VDC was judged to be only a weak teratogen with little primary effect on development.

I. INTRODUCTION

Vinylidene chloride (1,1-dichloroethylene, VDC) and vinyl chloride (1-chloroethylene, VCM) are monomeric intermediates used in the production of plastics. These plastics are employed in food packaging, pipe construction, and upholstery manufacturing. The 1974 production of VCM in the U.S. was 5.6 billion pounds.[1] Production figures for VDC are difficult to obtain; however, the current output may be estimated at about 1 billion pounds. Although VCM was associated with hepatic angiosarcoma in humans[2] there is relatively little information concerning the human toxicity of VDC, a structurally related compound. However, there are studies in animals concerning the toxicity of VDC.

A continuous 90-day inhalation exposure to VDC at a dose of 189 mg/m^3 (48 ppm) produced deaths in monkeys and guinea pigs, but not in dogs and rats.[3] A depression in growth occurred in all of the species tested. Morphological changes occurred in livers from monkeys, dogs, and rats, and kidneys from rats. Hepatic lesions consisted of fatty metamorphosis, focal necrosis, hemosiderin deposition, lymphocytic infiltration, bile duct proliferation, fibrosis, and pseudo-locule formation. The primary renal lesion was nuclear hypertrophy of the tubular epithelium. VDC and VCM both produced hepatic injury; however, VDC was a more potent hepatoxin.[4] In addition, the hepatic injury following VDC exposure differed from that produced by VCM. Exposure to VDC, in contrast to VCM, produced changes in the plasma membrane, mitochondria, and chromatin while sparing the endoplasmic reticulum. Biochemical signs of toxicity included increased activity of hepatic alkaline phosphatase and tyrosine transaminase; elevated plasma alanine transaminase and alkaline phosphatase activity; and reduced hepatic non-protein sulfhydryl groups and glucose-6-phosphatase activity.[5,6] The toxicity of VDC was influenced by the nutritional status of exposed animals[7] and the time of day the animals were exposed.[8] The increased sensitivity of these animals to VDC exposure was correlated with a reduction in the hepatic non-protein sulfhydryl concentration.[8]

Although the teratogenic potential of VDC has not been determined, studies on the effect of VCM exposure during development may be relevant. Mice, rats, and rabbits were exposed to 500 ppm of VCM during organogenesis.[9] Mice were more susceptible to VCM than either of the other two species. Maternal deaths (six deaths per 30 exposed) and a slightly reduced weight gain occurred in mice. In addition, there was a trend towards an increased incidence of resorptions, reduced pregnancy rate, and reduced fetal body weights. However, the incidence of external, soft tissue, and skeletal malformations was equivalent in control and exposed mice. The addition of 15% ethanol to the drinking water increased the toxicity of VCM in mice. In these mice, the maternal weight gain and pregnancy rate were further decreased

while the incidence of cleft palate and certain skeletal anomalies was significantly increased. VCM was judged, in this study, not to be teratogenic in mice, rats, and rabbits after daily 7-hr exposures at 500 ppm during organogenesis.

In summary, VDC and VCM are toxic in the adult. Although the effects of these compounds were similar, with the liver and kidney being the primary sites of toxicity, there were some differences in the types of lesions produced and the doses required to produce them. Although VCM was judged not to be a teratogen, there is no information available to evaluate the teratogenic potential of VDC.

The purpose of this study was to determine the effect of VDC exposure on development. The two parameters of development examined were the morphological development of rats and mice exposed to VDC in utero and the behavioral development of rats similarly exposed.

II. METHODS

A. Experimental Design

In this study, rats and mice were exposed to various concentrations of VDC in utero and their development was evaluated morphologically or behaviorally. The exposures were conducted in the trials listed below. In addition to the VDC-exposed groups, each trial also contained two control groups which were not exposed to VDC. One control group was given free access to feed while feed was restricted in the other control group. These two groups were included to provide a measure of normal development as well as development influenced by malnutrition. For the purpose of this report, the results are presented in the four parts indicated below. The morphological studies are contained in Part 1 (Trials I, II and III), Part 2 (Trial IV), and Part 3 (Trial V). The behavioral study is reported in Part 4 (Trial V).

Part	Trial	Animal	Concentration VDC (ppm)	Gestational Days Exposed
1	I	Mice	0, 15 and 300	6 to 16
		Rats	0, 15 and 300	6 to 16
	II	Mice	0, 57	6 to 16
		Rats	0, 57	6 to 16
	III	Mice	0, 30 and 144	6 to 16
		Rats	0, 449	6 to 16
2	IV	Mice	0 - 54 -	6 to 15
			0, 41, 54 and 74	8 to 15
			- 41, 54 -	10 to 15
			- - 54 -	12 to 15
3	V	Mice	0, 56, 81 and 112	6 to 9
			0, 56, 81 and 112	9 to 12
			0, 56, 81 and 112	12 to 15
			0, 56, 81 and 112	15 to 17
4	V	Rats	0, 56 and 283	8 to 20

B. Exposure

1. Chamber design: Five stainless steel cubical type animal exposure chambers were used in these studies. Three chambers were of 3.5 m^3 volume and two chambers were 4.5 m^3 in volume. The contaminants entered the inlet air stream and were mixed in a plenum at the top of each chamber. Air was exhausted at the bottom of the chamber.

2. Chamber air supply and flow rate: The air supply to the chambers was drawn from a stack on the roof of the building. Chamber air passed through a coarse filter and then over coils for heating, cooling and dehumidifying. Chamber temperature was maintained at 75°F ± 5° and humidity at 50% ± 10%.

Initially, air flow rates were measured at the inlet site of the chamber with pitot tubes connected to a magnehelic gauge. In later studies, air flow rates were measured at the exhaust side of the chambers with orifice plates connected to magnehelic gauges. Chamber air flows were calibrated using an Autotronics 100-ssx air flow transducer. Air flow rates were adjusted to maintain 10 to 15 air changes per hour in each of the chambers.

3. Chamber safety: All chambers were operated at a slight negative pressure (0.1-0.2 in. of water) to prevent escape of the contaminant into the room atmosphere. All air from the chambers traveled under negative pressure to an incinerator operated at a temperature of 1700° to 1800°F.

4. Generation of VDC vapor: Vinylidene chloride with a purity of 99% was obtained from the Aldrich Company. Two methods of generating VDC vapor were utilized. In Trials I to III, VDC was generated by heating to 37°C. Since VDC has a boiling point of 32°C, all gas lines and rotometers were heated to 40°C to prevent condensation. In Trials IV and V, the VDC vapor was generated by bubbling nitrogen into VDC contained in a glass flask. A stream of inlet air was directed through the flask and carried the VDC vapor into the plenum of the chamber. The rate of VDC generation was determined by the flow of nitrogen into the VDC flask. The nitrogen flow was controlled by the use of rotometers.

5. Chamber atmosphere monitoring: VDC was quantified with a Varian 2700 gas chromatograph equipped with a flame ionization detector and a stainless steel column (6 ft x 1/8 in.) packed with 0.4% Carbowax 1500 on Carbopak A. The injector, column, and detector temperatures were 135°C, 95°C and 170°C, respectively.

VDC standards were prepared by serial dilution (weight/volume) of VDC in carbon tetrachloride. The desired final concentrations in a 4 μl injection were determined by the following procedure:

$$\text{mg/liter } (\mu\text{g/ml}) = \frac{\text{ppm x mol wt}}{24450}$$

The amount of VDC (mol wt = 96.94) in a 1-ml sample from chambers that contain 10, 50 and 100 ppm VDC is 0.04, 0.20 and 0.40 μg, respectively. For 1 μl of standard, these same amounts would be obtained from solutions of 40, 200 and 400 mg/liter; but since 4 μl of standard were injected, the final dilutions of VDC in carbon tetrachloride were 10, 50 and 100 mg/liter. Each point on the calibration curve was the mean of three determinations.

Each chamber was equipped with 10 sampling ports on two sides of the chamber. Preliminary studies were conducted to determine the uniformity of the concentration of the test material at different points in the chamber. The concentration at the various sampling points was compared to a reference point in the center of the chamber. It was found that the average concentration at the various points was $\pm$ 3% of the desired chamber concentration. The concentration at the reference point was 98.4% to 100.4% of the average chamber concentration. Therefore, the chambers were routinely sampled at the reference point for determination of chamber concentrations. The chamber samples were drawn through polyethylene sample lines into a gas tight syringe. One milliliter samples of chamber air were injected into the gas chromatograph for determination of chamber concentration.

In Trials I to III, the chamber concentrations were determined by measurement of peak heights on a recorder connected to the gas chromatograph. The chamber concentration was determined by extrapolation from a standard curve prepared at the beginning of each day using VDC standards. In Trials IV and V, a Varian CDS-111 chromatography data system was used to determine chamber concentrations. The CDS-111 calculates chamber concentration by integration of peak areas. The CDS-111 was calibrated with VDC standards at the beginning of each day and provided a direct printout of chamber concentration in parts per million.

C. Animals

CD rats and CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were housed in our animal quarters for at least 7 days prior to use. These quarters were maintained at 24 $\pm$ 2°C with a relative humidity of 50 $\pm$ 10% and a 7 AM to 7 PM photoperiod. Rats were generally housed two per cage and mice six per cage. Animals were given free access to rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) and tap water before, during, and after exposure, except where indicated. During the exposure period, animals were housed in stainless steel cages with wire mesh bottoms and their feed and water were changed daily. When feed consumption was determined, the animals were given powdered rodent chow in stainless steel feeders (Hoeltge, Cincinnati, Ohio) which were designed to minimize spillage. Animals in the feed-restricted groups were given their feed in round glass jars in order to provide better access to their limited amount of feed. Initially, attempts were made to feed these animals the same amount of feed consumed by the group exposed to the high concentration of VDC. Since we did not achieve a high degree of accuracy in this regard, the group has been termed a feed-restricted group rather than a pair-fed group. The weight gain and, in most cases, the feed consumption for this group is presented in the appropriate table.

D. Protocol for Morphological Evaluation

1. Mating: Sexually mature virgin female mice were housed overnight with a proven male breeder. The next morning the females were examined for evidence of copulation as demonstrated by the presence of a vaginal plug. Sexually mature virgin female rats were examined by vaginal lavage late in the afternoon for signs of proestrus (75 to 90% nucleated epithelial cells). Females in proestrus were placed overnight with an experienced male. The following morning, females were examined for the presence of sperm. The day on which evidence of mating was discovered was identified as being day 0 of gestation.

2. Exposure: Groups of plug-positive mice and sperm-positive rats were exposed to various concentrations of VDC as indicated in the above section on experimental design. The treatments were administered for 22 to 23 hr a day. The exposures were terminated for 1 to 2 hr in the morning so that animals could be added to or removed from the chamber and their feed and water could be changed.

3. Examination: Mice and rats were sacrificed by CO_2 anesthesia on gestational days 17 and 20, respectively. A laparotomy was performed and the number and position of live, dead, and resorbed fetuses were recorded. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed, weighed, and examined for gross anomalies.[10/] One-half of the fetuses from each litter were fixed in Bouins solution and examined for soft tissue anomalies using a free hand slicing technique.[10/] The remaining fetuses from each litter were processed for skeletal examination. Fetuses were fixed in 70% alcohol, eviscerated, and stored in 1% KOH for 2 days. Afterwards, they were stained with alizarin red.[11/] The skeletons were examined for anomalies after differential decolorization.

E. Protocol for Behavioral Evaluation

1. Mating: Rats were mated as described above.

2. Exposure: Groups of sperm-positive rats were exposed to 0, 56 and 283 ppm of VDC from gestational days 8 to 20, as indicated above.

3. Examination: Pregnant females, exposed to 0 ppm (control), 0 ppm feed restricted, 56 ppm or 283 ppm of VDC from gestational days 8 to 20, were examined for litters at 8 AM, noon, 5 PM and 10 PM. Those litters born between 10 PM and 9 AM, and those born between 8 AM and noon were tested on the behavioral tasks starting on the morning following their birth (day 1). Those born between noon and 5 PM were tested first on the afternoon of the following day (day 1). Those litters born between 5 PM and 10 PM were not used for preweaning tests. All groups, first tested in the morning, were tested in the morning from then on. The same procedure was used for the afternoon groups.

On day 1, the pups were sexed and weighed and each litter given a coded number. Occasionally, pups were transferred to other litters within age and treatment groups to balance the sexes. All litters were culled to eight pups with equal numbers of each sex. Body weights were obtained on alternate days until weaning at 21 days of age.

From each litter, one male and one female were marked by tail clipping for inclusion into the preweaning behavioral testing. All testing was done with the experimenter ignorant of the treatment group to which the pup belonged.

a. Preweaning tests:

(1) Surface righting:[12,13] This test started on day 1. The pup was placed on its back and the time required for the pup to turn over onto all four feet was recorded. The test was conducted three times and the average recorded as the score for the day. Testing continued until three daily average times were 1 sec or better. An upper limit of 30 sec was applied.

(2) Pivoting:[13] Testing started on day 4. Young animals frequently move in circles with one rear foot held in place; this is called pivoting. The total time spent pivoting during a 3-min test was recorded. Daily testing continued until the pups did not show any pivoting during 3 consecutive days.

(3) Auditory startle:[12,13] Testing started on day 10 and continued until a good response was observed on 3 consecutive days. The pups were placed in a sound attenuating box and then a sharp noise was produced over the animal by snapping a device constructed from a mouse trap. The startle response consisted of sudden flexion and head turning of the pup following the noise.

(4) Bar holding:[13] This test was started on the 7th day. The pup was placed on a 1/4-in. horizontal aluminum bar. If the pup held onto the bar, the time it hung on was recorded in seconds up to a maximum of 15 sec. If the pup did not hang on the bar it fell onto a pile of foam rubber. Pups that fell off immediately were given two other trials. Testing continued until three consecutive daily scores of 15 sec were recorded, or until the pup was able to negotiate the bar and climb off.

(5) Righting in air:[12,13] This test was started on day 12 and concluded upon three consecutive daily rightings. When held with its stomach up and dropped, a mature rat will right itself in air and land on its feet. Pups were dropped from a height of 50 cm onto a thick, soft bed of foam rubber.

(6) Visual placing:[12,13] This test started on day 12 and concluded when placing was present on 3 consecutive days. The test consisted of grasping the pup by the flank and moving it toward the edge of a table. A mature rat will reach out for the edge as its body approaches it.

(7) Swimming:[14,15] Swimming tests started on day 7 and continued until swimming ability was judged to be mature. Testing was conducted in a 20-gal aquarium containing approximately 15 gal of water at 20°C. Judgements were based on a rating scale of 0 to 4 which reflected the extent to which the animal kept its nose and ears out of the water.

Pluses and minuses were also used to score the use of the forefeet or hind feet in locomotion. An immature rat swims with its nose and ears submerged and paddles with its front feet. A mature rat keeps it nose and ears out of the water and kicks primarily with its hind feet only.

(8) Physical maturation: In addition to the preweaning behavioral tests, indicies of physical maturation were recorded daily. These included detachment of the pinnae (from day 1); incisor eruption (from day 5); and opening of the eyes (from day 7). Also, body weights of the eight pups in each litter were obtained on alternate days.

b. Activity test: At about 3 weeks of age, rats within dose and sex were weaned into groups of three. They were used for measuring activity levels, starting when they were 95 ± 25 days of age. The apparatus was a resident maze[16] (Figure 1) which contained six infrared sensors. Five of these mazes were used simultaneously. Groups of three rats were placed into the maze and left there for 70 hr. Each time a rat crossed an infrared beam a count was registered on a counter. Every 2 hr, a timer caused the counter to print the total number of counts accumulated and then reset to zero.

In all, there were seven groups of males and seven groups of females for each dose level. They were from 14 to 20 different litters. Testing was in a counterbalanced order for both groups and apparatuses. The sexes were tested on alternate days. Due to equipment malfunction, the data for the last group of males was lost, leaving seven groups (21 rats) of females and six groups (18 rats) of males for the data analysis.

F. Statistics

All quantitative data were reported as the mean ± S.E. and analyzed for significance by either parametric or nonparametric tests. The parametric tests used in this study were the analysis of variance followed by Tukey's omega procedure[17] for the morphological data and Duncan's multiple range test[17] for the behavioral data. The level of significance was chosen as $p < 0.05$. The non-parametric test used in this study was the two sample rank test[18] with a level of significance selected as $p < 0.05$ (one tail test). Enumeration data were analyzed with the Fisher's exact probability test.[19]

III. RESULTS

A. Chamber Concentrations of VDC

The standard curve for measuring VDC concentrations was initially prepared by injecting varying volumes of a VDC standard, prepared in chloroform, into the gas chromatograph. This procedure produced a non-linear response

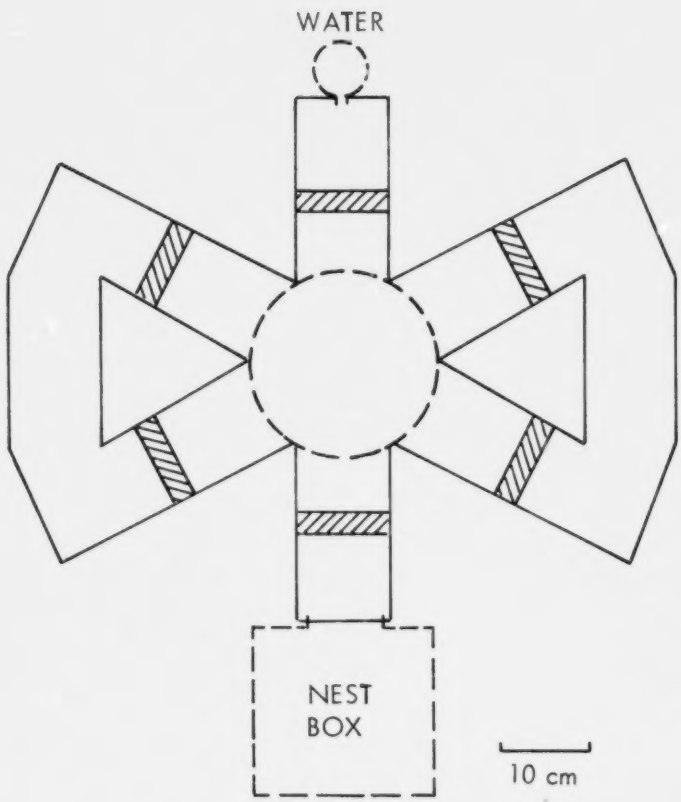

Figure 1 - Diagram of Activity Maze. The sides and nest box are constructed of galvanized steel and rests on an expanded aluminum floor. The runway walls are 10 cm high and the nest box walls are 25 cm high. Crossbars indicate locations of infrared beams. The runways are covered by a sheet of clear plexiglas with a raised dome in the center.[5/]

which prevented the accurate measurement of chamber concentrations. This problem was corrected by injecting a constant volume of standards prepared in carbon tetrachloride.

The chamber concentrations of VDC used during the various trials are presented in Table 1. As the result of our early problems in obtaining a linear standard curve it was not possible to accurately measure chamber concentrations in Trial I by gas chromatography. Therefore, the concentrations for this trial were calculated from mass balance data (i.e., the number of air changes per hour and the amount of VDC consumed per day). In subsequent trials, the chamber concentrations were measured using gas chromatography. For Trials II to IV, the concentrations were measured, on the average, four to five times a day from 8 AM to 5 PM. In the evening, the flow meters were monitored in an effort to control the chamber concentration. During Trial V the VDC concentrations were measured, on the average, 10 to 11 times a day at 2-hr intervals. For Trials II to V, the chamber concentration for a given day was determined by averaging the individual values determined during that day.

B. Morphological Studies on Mice and Rats

1. Mice and rats exposed from gestational days 6 to 16 (Part 1):

a. General observations: Mice were exposed to 0, 15, 30, 57, 144 and 300 ppm of VDC from gestational day 6 to 16. The effects of this treatment on maternal welfare and reproduction are presented in Table 2. A reduced ratio of pregnant to exposed mice occurred in the group exposed to 30 and 57 ppm of VDC. Increased mortality in pregnant mice was observed at VDC concentrations of 114 ppm and above. In addition, increased mortality was observed in pregnant mice in the feed restricted group. Increased mortality also occurred in non-pregnant mice exposed to 144 and 300 ppm of VDC. Feed consumption and weight gain during exposure were reduced in the groups exposed to 30 and 57 ppm of VDC as well as the feed restricted group. The group exposed to 57 ppm of VDC had an increased number of dams with complete resorptions. Viable fetuses were only produced in the control group, the group exposed to 15 ppm of VDC, and the feed restricted group. Fetal body weights were reduced only in the feed restricted group.

Rats were exposed to 0, 15, 57, 300 and 449 ppm of VDC from gestational days 6 to 16. The effects of this treatment on maternal welfare and reproduction are shown in Table 3. A reduced ratio of pregnant to exposed rats occurred in the group exposed to 300 ppm of VDC. Deaths occurred in pregnant rats exposed to VDC concentrations of 57 ppm and above. The weight gain during exposure was reduced in all the groups exposed to VDC.

TABLE 1

CHAMBER CONCENTRATIONS OF VINYLIDENE CHLORIDE DURING VARIOUS TRIALS

Trial	Vinylidene Chloride (ppm) Mean	Range[c/]
I	15[a/]	--
	300	--
II	57 ± 2 (14)[b/]	(51-81)
III	30 ± 1 (10)	(25-38)
	144 ± 12 (3)	(131-168)
	449 ± 5 (14)	(415-478)
IV	41 ± 1 (18)	(34-52)
	54 ± 2 (17)	(46-61)
	74 ± 2 (18)	(51-86)
V	56 ± 1 (18)	(52-68)
	81 ± 1 (15)	(74-91)
	112 ± 2 (13)	(97-120)
	283 ± 4 (18)	(228-303)

a/ Mean calculated from mass balance data.
b/ Mean ± S.E. for the indicated number of daily values determined by gas chromatography.
c/ Range of daily values.

TABLE 2

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 16 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)						
	0[c]	15	30	57	144	300	0[d]
Number Exposed	65	23	19	21	18	15	20
Pregnant	38	16	2[e]	5[e]	8	8	9
Alive	38	16	2	4	0[e]	0[e]	7[e]
Non-Pregnant	27	7	17	15	10	7	11
Alive	27	7	17	14	0[e]	0[e]	11
Body Weight Change[a]							
During exposure	11.7 ± 1.5	11.0 ± 1.0	1	10.0 ± 2.0[f]	--	--	5.0 ± 2.0[f]
After exposure	6.2 ± 0.7	7.3 ± 1.2	1	7.0 ± 1.7	--	--	5.1 ± 1.7
Feed Consumption[b]	5.3 ± 0.2	6.0 ± 0.9	4.2 ± 0.1[f]	3.3 ± 0.3[f]	--	--	3.6[f]
Pregnant Surviviors	38	16	2	4	0	0	7
Implants/Dam	11.1 ± 0.4	10.7 ± 0.6	4.0	6.8 ± 2.3	--	--	8.6 ± 1.7
Viable fetuses (%)	82 ± 5	87 ± 6	0	0[f]	--	--	42 ± 18[f]
Dead fetuses (%)	1 ± 0	2 ± 1	0	0	--	--	0
Early resorptions (%)	11 ± 4	8 ± 6	100	100[f]	--	--	46 ± 19
Late resorptions (%)	3 ± 1	5 ± 2	0	0	--	--	12 ± 12
Dams with complete resorptions	3	0	2	4[e]	--	--	3[e]
Live Litters	35	16	0	0	0	0	4
Fetuses/Dam	9.8 ± 0.4	10.3 ± 0.4	--	--	--	--	9.0 ± 2.3
Males (%)	52 ± 4	54 ± 3	--	--	--	--	54 ± 7
Fetal Weight (gm)	1.2 ± 0.0	1.3 ± 0.1	--	--	--	--	0.9 ± 0.1[g]

a/ Gm/animal/interval for pregnant rats.
b/ Gm/animal/day during exposure for pregnant rats.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (two sample rank test).
g/ Significantly different from control (Tukey's omega procedure).

TABLE 3

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 16 ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Vinylidene Chloride (ppm)					
	0[c]	15	57	300	449	0[d]
Number Exposed	58	18	20	18	18	18
Pregnant	50	14	16	9	15	14
Alive	50	13	13[e]	6[e]	8[e]	14
Non-pregnant	8	4	4	9	4	4
Alive	8	3	4	8	3	4
Body Weight Change[a]						
During exposure	44 ± 23	-28 ± 8[f]	-64 ± 6[f]	-83 ± 13[f]	-62 ± 19[f]	-61 ± 5[f]
After exposure	52 ± 4	91 ± 6[f]	80 ± 10[f]	81 ± 10[f]	49 ± 6	91 ± 8[f]
Feed Consumption[b]	19.0 ± 0.8	15.3 ± 3.6	7.0 ± 1.1[f]	4.8 ± 0.9[f]	5.6 ± 0.5[f]	3.5[f]
Pregnant Survivors	50	13	13	6	8	14
Implants/Dam	13.3 ± 0.3	12.5 ± 0.5	12.3 ± 1.0	9.8 ± 1.9	12.9 ± 1.8	13.0 ± 0.3
Viable fetuses (%)	98 ± 1	95 ± 2	51 ± 14[f]	93 ± 4	23 ± 15[f]	98 ± 1
Dead fetuses (%)	0	0	0	0	0	0
Early resorptions (%)	2 ± 1	5 ± 2	49 ± 14[f]	7 ± 4	64 ± 18[f]	2 ± 1
Late resorptions (%)	0.4 ± 0.2	0	0	0	13 ± 13	0
Dams with complete resorptions	0	0	6[c]	0	6[c]	0
Live Litters	50	13	7	6	2	14
Fetuses/Dam	13.0 ± 0.4	11.9 ± 0.6	12.9 ± 0.9	9.2 ± 1.9[f]	13.5 ± 0.5	12.8 ± 0.3
Males (%)	51 ± 2	53 ± 4	46 ± 5	49 ± 11	51 ± 13	53 ± 3
Fetal weight (gm)	3.6 ± 0.1	3.4 ± 0.1	3.1 ± 0.1[g]	3.0 ± 0.1[g]	2.7 ± 0.2[g]	3.0 ± 0.1[g]

a/ Gm/animal/interval for pregnant rats.
b/ Gm/animal/day during exposure for pregnant rats.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (Tukey's omega procedure).

The weight gain after treatment was increased in all of the VDC exposed groups except the group exposed to 449 ppm. Feed consumption was reduced in the groups exposed to VDC concentrations of 57 ppm and above. Feed consumption was also reduced in the feed restricted group. The percent of implants with viable fetuses was reduced in the groups exposed to 57 and 449 ppm of VDC. In addition, the ratio of dams with complete resorptions to pregnant survivors was increased in these groups. The resorptions which occurred in these two groups were classified as being early resorptions. Fetal body weights were reduced in the groups exposed to 57, 300 and 449 ppm of VDC as well as the feed restricted group. The number of fetuses/dam was reduced in the group treated with 300 ppm of VDC.

b. Anomalies: There was neither a significant increase in gross anomalies nor a pattern of gross anomalies in either mice or rats following exposure to VDC.

The soft tissue anomalies in mice and rats following exposure to VDC are presented in Tables 4 and 5, respectively. There was no significant increase in anomalies from mice exposed to 15 ppm of VDC. However, in rats there was an increase in the incidence of lateral ventricle hydrocephalus in rats exposed to 15 and 57 ppm of VDC. A similar incidence of this anomaly also occurred in the group exposed to 300 ppm; however, this level was not statistically significant.

Skeletal anomalies observed in mice and rats exposed to VDC are reported in Tables 6 and 7, respectively. Ossification problems with the incus and sternebrae occurred in mice exposed to 15 ppm of VDC. A similar incidence of unossified incus also occurred in the feed restricted group. Rats from the groups exposed to 15, 57 and 300 ppm of VDC had ossification problems with the sternebrae.

2. Mice exposed for various intervals ending on gestational day 15 (Part 2):

a. General observations: Mice were exposed to 0, 41, 54 and 74 ppm of VDC for 3 to 9 day intervals that ended on gestational day 15. The effects of these concentrations of VDC on maternal welfare and reproduction following a 7-day exposure, which started on gestational day 8, are presented in Table 8. Significant mortality occurred in pregnant mice exposed to 74 ppm of VDC. A reduced weight gain during exposure occurred both in the VDC treated groups and the feed restricted group. However, this effect was not as dramatic in the latter group. At the end of the exposure period, the weight change was significantly increased for all of these groups with the exception of the group exposed to 74 ppm of VDC. In addition, dams in this group had a significantly reduced number of implants and percent of these implants with viable fetuses. All of the implants in the surviving dams were characterized as being early resorptions.

TABLE 4

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 16 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Vinylidene Chloride (ppm)		
	0[a]	15	0[b]
Number of			
Litters inspected	7	15	3
Fetuses inspected	36	73	13
Soft Tissue Anomalies			
Hydrocephalus: lateral ventricle	0±0[c]	2.6±1.8	0±0
lateral ventricle slight	0±0	2.3±1.6	0±0
Nasal passage occluded	0±0	1.9±1.9	0±0
Microphthalmia	0±0	3.6±2.4	0±0
Palate: cleft	0±0	1.7±1.7	6.7±6.7
Liver: small	0±0	1.7±1.7	0±0
Kidney: hydronephrosis	0±0	5.8±2.6	0±0

a/ Control group.

b/ Feed restricted group.

c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

TABLE 5

EFFECT OF VINYLIDENE CHLORIDE ON GESTATIONAL DAYS 6 TO 16 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN RATS

	Vinylidene Chloride (ppm)				
	0[a]	15	57	300	0[b]
Number of					
Litters inspected	50	13	7	6	14
Fetuses inspected	211	75	41	27	85
Soft tissue anomalies					
Hydrocephalus: lateral ventricle	2.5±2.1[c]	7.3±2.3[d]	15.1±6.3[d]	33.3±21.1	0±0
lateral ventricle, slight	0.3±0.3	1.3±1.3	0±0	0±0	2.4±1.6
third ventricle	0.4±0.4	0±0	0±0	0±0	0±0
fourth ventricle	2.5±2.5	0±0	0±0	0±0	0±0
Nasopharyngeal canal occluded	0.3±0.3	0±0	0±0	0±0	0±0
Nasal passage occluded	1.0±0.6	0±0	0±0	0±0	0±0
Palate: short	0±0	2.4±1.6	0±0	0±0	0±0
Lung: agenesis	0.3±0.3	0±0	0±0	0±0	0±0
small	0±0	0±0	4.9±3.2	0±0	0±0
deflated	1.1±0.7	0±0	0±0	0±0	0±0
Small bronchus opening	0.3±0.3	0±0	0±0	0±0	0±0
Liver: small	0±0	0±0	0±0	8.3±8.3	0±0
Kidney: hydronephrosis	2.6±1.1	5.5±2.5	0±0	25.0±17.1	2.0±2.0
small	0±0	1.5±1.5	0±0	6.1±3.9	0±0
ectopic	0.3±0.3	0±0	0±0	0±0	0±0
Distended urinary bladder	2.5±1.5	0±0	0±0	0±0	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control in respective trial (two-sample rank test).

TABLE 6

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 16 ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Vinylidene Chloride (ppm)		
	0[a/]	15	0[b/]
Number of			
Litters inspected	7	15	3
Fetuses inspected	38	76	15
Skeletal anomalies			
Skull collapsed: marked	0±0	3.2±1.7	0±0
Nasal bones elevated	2.8±2.8	4.5±2.6	0±0
Premaxillary process: incompletely ossified	0±0	0±0	5.6±5.6
Supraoccipital: incompletely ossified	6.4±4.2	12.2±6.2	57.2±20.3
extra ossification	0±0	2.3±1.6	0±0
Incus: unossified	0±0	21.4±8.0[d/]	86.1±7.3[d/]
Mandible: short	0±0	1.1±1.1	0±0
incompletely ossified	0±0	4.1±2.8	0±0
Sternebrae: ossified normally	35.0±9.1	37.3±6.2	19.4±10.0
unossified	0±0	1.7±1.7	22.2±22.2
incompletely ossified	0±0	13.6±4.4[d/]	45.0±23.0
split	19.6±7.5	28.1±7.6	44.4±29.4
malaligned	29.9±9.7	25.6±4.8	38.3±7.3
extra ossification	8.6±8.6	12.4±7.2	0±0
Lateral curvature of spine	0±0	1±1	0±0
Centri: ossified normally	85.7±14	100±0	100±0
Ribs: extra	20.7±1.07	31.1±9.1	83.3±16.7
Paws: unossified	2.8±2.8	1.1±1.1	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control (two-sample rank test).

TABLE 7

EFFECT OF VINYLIDENE CHLORIDE ON GESTATIONAL DAYS 6 TO 16 ON THE INCIDENCE OF SKELETAL ANOMALIES IN RATS

Number	Vinylidene Chloride (ppm)				
	0[a]	15	57	300	0[b]
Number of					
Litters inspected	48	13	7	6	14
Fetuses inspected	323	79	48	27	94
Skeletal anomalies					
Skull collapsed	1.9±0.7[c]	5.9±4.5	4.2±2.7	20.0±16.3	0±0
slight	0.4±0.4	4.4±3.2	3.8±2.5	0±0	0±0
marked	0.3±0.3	0±0	0±0	0±0	0±0
Nasal bones: elevated	0.3±0.3	0±0	0±0	0±0	0±0
Premaxillary: malaligned	4.4±2.1	0±0	0±0	0±0	0±0
Occipital fontanel: enlarged	1.2±0.8	0±0	2.4±2.4	0±0	0±0
Parietals: incompletely ossified	0.3±0.3	1.0±1.0	0±0	0±0	0±0
Interparietals: incompletely ossified	1.6±1.0	1.3±1.3	7.1±5.0	0±0	0±0
Supraoccipital: incompletely ossified	0±0	0±0	4.4±2.9	8.3±8.3	0±0
Squamosal: split	1.0±1.0	0±0	0±0	0±0	0±0
Mandibles: shortened	0.8±0.5	0±0	0±0	0±0	0±0
incompletely ossified	0±0	1.0±1.0	0±0	0±0	0±0
malaligned	6.4±2.1	0±0	21.4±9.5	0±0	0±0
Hyoid bone: unossified	2.7±1.0	0±0	3.6±3.6	0±0	0±0
incompletely ossified	0.3±0.3	0±0	0±0	0±0	0±0
malaligned	25.4±3.8	10.5±5.3[d]	4.2±2.7[d]	0±0[d]	21.3±5.2
Sternebrae: ossified normally	25.7±5.0	51.4±7.7	72.4±7.5[d]	68.2±12.5	50.1±9.3
unossified	44.8±3.5	50.1±7.5	60.5±8.1	59.8±16.6	50.3±5.8
incompletely ossified	5.0±2.2	28.1±9.7	6.1±6.1	19.4±13.9	15.0±5.7
split	5.7±1.9	18.3±3.6[d]	6.3±3.1	30.7±15.8	11.2±3.4
malaligned	0±0	0±0	0±0	11.1±8.2	0±0
Lateral curvature to spine	69.9±4.0	72.9±8.2	71.8±10.8	51.8±15.1	71.6±6.4
Centri: ossified normally	25.3±3.6	24.6±7.3	25.9±8.9	48.2±15.1	25.2±5.2
lobed	5.9±1.6	3.7±1.9	2.4±2.4	0±0	4.3±2.4
split	0.3±0.3	0±0	0±0	0±0	0±0
Ribs: unossified	9.3±2.4	0±0	4.4±2.9	0±0	12.4±4.7
extra	1.7±1.7	0±0	0±0	0±0	0±0
Pelvis: unossified	1.7±1.7	1.3±1.3	0±0	0±0	0±0
incompletely ossified	0±0	2.2±2.2	0±0	0±0	0±0
Paws: incompletely ossified					

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control in respective trial (two sample rank test).

TABLE 8

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 8 TO 15 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)				
	0[b]	41	54	74	0[c]
Number Exposed	24	22	20	17	17
Pregnant	17	21[d]	12	9	13
Alive	17	21	12	3[d]	13
Non-pregnant	7	1	8	8	4
Alive	7	1	8	4	4
Body Weight Change[a]					
During exposure	12.5 ± 0.4	2.8 ± 1.1[e]	1.3 ± 1.5[e]	-8.7 ± 1.5[e]	10.2 ± 0.4[e]
After exposure	4.4 ± 0.9	11.4 ± 1.1[e]	12.2 ± 1.2[e]	-0.3 ± 0.7[e]	7.5 ± 0.4[e]
Pregnant Survivors	17	21	12	3	13
Implants/dam	10.8 ± 0.4	10.0 ± 0.7	10.3 ± 0.9	6.3 ± 1.9[e]	10.6 ± 0.5
Viable fetuses (%)	92 ± 2	75 ± 8	83 ± 8	0[e]	90 ± 2
Dead fetuses (%)	0	0.3 ± 0.3	1.5 ± 1.5	0	0.6 ± 0.6
Early resorptions (%)	4 ± 1	21 ± 9	11 ± 8	100 ± 0[e]	5 ± 2
Late resorptions (%)	4 ± 1	4 ± 1	5 ± 2	0	3 ± 1
Dams with complete resorptions	0	4	1	3[d]	0
Live Litters	17	17	11	0	13
Fetuses/dam	10.1 ± 0.5	10.5 ± 0.3	9.7 ± 0.8	-	9.6 ± 0.5
Males (%)	52 ± 5	43 ± 4	44 ± 4	-	53 ± 4
Fetal weight (gm)	1.13 ± 0.3	1.08 ± 0.05	1.06 ± 0.03	-	1.23 ± 0.03

a/ Gm/animal/interval for pregnant mice.
b/ Control group.
c/ Feed restricted group.
d/ Significantly different from control (Fisher's exact probability test).
e/ Significantly different from control (two sample rank test).

The effects of 41 ppm of VDC on maternal welfare and reproduction, following both a 7- and 5-day exposure ending on gestational day 15, are presented in Table 9. The control and VDC exposed group from Table 8 are included for comparison. The weight gain during exposure was reduced for both VDC exposed groups relative to the appropriate control values. However, the weight change after exposure was increased only in the group with the more prolonged exposure. Dams in the group exposed to VDC from gestational days 10 to 15 had a reduced percent of viable fetuses and an increase both in the percent of early resorptions and the number of dams with complete resorptions. In addition, the number of fetuses/dam was also reduced in this group.

The effects of 54 ppm of VDC on maternal welfare and reproduction following exposure periods of 9, 7, 5 and 3 days ending on gestational day 15, are presented in Table 10. A significant increase in mortality occurred in the group whose exposure started on gestational day 6. The weight gain during exposure was reduced for all of the VDC treated groups relative to the appropriate control values. An increased weight gain after exposure occurred only in the group of mice whose treatment period started on gestational day 8. All of the surviving dams exposed from gestational days 6 to 15 had complete resorptions. Dams treated for 5 days starting on gestational day 10 had a reduced percent of viable fetuses, an increased percent of both early and late resorptions, and an increased number of dams with complete resorptions. In addition, fetuses/dam and fetal body weight were reduced in this group. Similar effects were observed in dams treated for 3 days starting on gestational day 12; however, there was no effect on the incidence of early resorptions.

b. Anomalies: The gross anomalies observed in fetuses from dams exposed to 41 and 54 ppm of VDC are presented in Table 11. Immature skin, which was defined as being a sticky, poorly developed integument, hematoma, and exencepaly were observed. Immature skin and hematoma occurred in both the control and VDC exposed groups and did not increase in a dose related manner. Exencepaly, in contrast, occurred only in the group exposed to 81 ppm of VDC.

The effects of exposing pregnant mice to 41 and 54 ppm of VDC on the incidence of soft tissue anomalies are presented in Tables 12 and 13, respectively. Although anomalies were observed in mice exposed to 41 ppm of VDC from gestational days 8 to 15 and 10 to 15 there was no significant increase in the incidence of these anomalies relative to the control group. However, an increased incidence of cleft palate occurred in mice exposed to 54 ppm of VDC from gestational days 10 to 15 and 12 to 15 but not 8 to 15.

TABLE 9

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE AT 41 PPM ON MATERNAL WELFARE AND REPRODUCTION IN MICE

VDC (ppm)	0 [b]	41	41
Days Exposed	8 to 15	8 to 15	10 to 15
Number Exposed	24	22	20
Pregnant	17	21	18
Alive	17	21	17
Non-pregnant	7	1	2
Alive	7	1	2
Body Weight Change [a]			
During exposure	12.5 ± 0.4	2.8 ± 1.1 [c]	-4.0 ± 0.7 [c]
After exposure	4.4 ± 0.9	11.4 ± 1.1 [c]	5.7 ± 1.0
Pregnant Survivors	17	21	17
Implants/dam	10.8 ± 0.4	10.0 ± 0.7	9.3 ± 0.9
Viable fetuses (%)	92 ± 2	75 ± 8	29 ± 8 [c]
Dead fetuses (%)	0	0	1 ± 1
Early resorptions (%)	4 ± 1	21 ± 9	64 ± 9 [c]
Late resorptions (%)	4 ± 1	4 ± 4	6 ± 3
Dams with complete resorptions	0	4	8 [d]
Live Litters	17	17	9
Fetuses/dam	10.1 ± 0.5	10.5 ± 0.3	5.2 ± 1.0 [c]
Males (%)	52 ± 5	43 ± 4	45 ± 11
Fetal weight (gm)	1.13 ± 0.03	1.08 ± 0.05	1.20 ± 0.20

a/ Gm/animal/interval for pregnant mice.
b/ Control group.
c/ Significantly different from control (two sample rank test).
d/ Significantly different from control (Fisher's exact probability test).

TABLE 10

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE AT 54 PPM ON MATERNAL WELFARE AND REPRODUCTION IN MICE

VDC (ppm)	0[b]	54	54	54	54
Days Exposed	6 to 15	6 to 15	8 to 15	10 to 15	12 to 15
Number Exposed	24	22	20	24	13
Pregnant	17	7	12	17	11
Alive	17	3[c]	12	12	10
Non-pregnant	7	15	8	7	2
Alive	7	11	8	6	1
Body Weight Change[a]					
During exposure	12.5 ± 0.4	-4.7 ± 0.9[d]	1.3 ± 1.5[d]	-3.6 ± 0.8[d]	-8.1 ± 0.7[d]
After exposure	4.4 ± 0.9	1.3 ± 0.7	12.2 ± 1.2[e]	6.2 ± 0.5	2.7 ± 0.5
Pregnant Survivors	17	3	12	12	10
Implants/dam	10.8 ± 0.4	3.3 ± 0.9[e]	10.3 ± 0.9	10.5 ± 0.8	10.2 ± 0.8
Viable fetuses (%)	92 ± 2	0	83 ± 8	23 ± 9[e]	32 ± 9[e]
Dead fetuses (%)	0	0	2 ± 2	0	10 ± 10
Early resorptions (%)	4 ± 1	100 ± 0[e]	11 ± 8	23 ± 8[e]	9 ± 5
Late resorptions (%)	4 ± 1	0	5 ± 2	54 ± 13[e]	49 ± 12[c]
Dams with complete resorptions	0	3	1	6[c]	4[c]
Live Litters	17	0	11	6	6
Fetuses/dam	10.1 ± 0.5	-	9.7 ± 0.8	5.2 ± 1.8[e]	5.0 ± 0.6[e]
Males (%)	52 ± 5	-	44 ± 4	51 ± 13	47 ± 6
Fetal weight (gm)	1.13 ± 0.03	-	1.06 ± 0.03	0.93 ± 0.05[e]	0.81 ± 0.06[e]

a/ Gm/animal/interval for pregnant mice.
b/ Control group.
c/ Significantly different from control (Fisher's exact probability test).
d/ Significantly different from control (Tukey's omega procedure).
e/ Significantly different from control (two sample rank test).

TABLE 11

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON THE INCIDENCE OF GROSS ANOMALIES IN MICE

VDC (ppm)	Gestational Days Exposed	Gross Anomalies		
		Immature Skin	Hematoma	Exencephaly
0[a]	8 to 15	1.2±1.2[c]	4.4±1.6	0±0
41	8 to 15	18.7±9.4	2.3±1.1	0±0
54	8 to 15	6.5±4.6	0±0	5.3±3.2
	10 to 15	0±0	0±0	0±0
	12 to 15	0±0	0±0	0±0
0[b]	8 to 15	1.5±1.5	1.5±1.5	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

TABLE 12

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE AT 41 PPM ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

VDC (ppm) / Days Exposed	0[a] 6 to 15	41 8 to 15	41 10 to 15	0[b] 8 to 15
Number of				
Litters inspected	17	17	9	13
Fetuses inspected	32	86	22	62
Soft tissue anomalies				
Hydrocephalus: lateral ventricle	0±0[c]	9.8±6.4	11.1±11.1	0±0
third ventricle	0±0	12.6±6.6	0±0	0±0
fourth ventricle	5.3±3.2	6.4±3.3	0±0	1.9±1.9
Olfactory bulb with depression	0±0	1.5±1.5	0±0	0±0
Nasal cavity occluded	1.0±1.0	0±0	11.1±11.1	2.6±1.7
Nasopharyngeal canal occluded	2.0±1.3	2.5±1.7	0±0	0±0
Nasal passage occluded	4.5±3.6	5.3±2.5	8.3±6.0	14.4±5.0
Cleft palate	0±0	1.5±1.5	10.2±7.6	0±0
Deflated lung	1.2±1.2	2.4±1.6	0±0	0±0
Kidney: hydronephrosis	1.2±1.2	2.8±2.8	2.9±2.0	1.3±1.3
small	0±0	0±0	16.7±11.8	0±0
cortex solidified	0±0	6.3±2.5	5.6±5.6	5.8±3.0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis

TABLE 13

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE AT 54 PPM ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

VDC (ppm)	0[a]	54	54	54[b]
Days Exposed	6 to 15	8 to 15	10 to 15	12 to 15
Number of				
Litters inspected	17	11	6	6
Fetuses inspected	82	54	16	13
Soft tissue anomalies				
Hydrocephalus: lateral ventricle	0±0[b]	5.3±3.6	0±0	0±0
third ventricle	0±0	4.5±3.0	0±0	0±0
fourth ventricle	5.3±3.2	14.5±4.9	11.7±8.3	5.6±5.6
Temporal artery hemorrhage	0±0	3.8±2.6	0±0	0±0
Nasal cavity occluded	1.0±1.0	1.5±1.5	0±0	8.3±8.3
Nasopharyngeal canal occluded	2.0±1.3	1.3±1.3	16.7±16.7	0±0
Nasal passage occluded	4.5±3.6	14.5±7.7	0±0	16.7±16.7
Micropthalmia	0±0	7.6±6.1	0±0	0±0
Ectopic eye	0±0	2.3±2.3	0±0	0±0
Cleft palate	0±0	7.1±3.8	31.7±16.4[c]	44.4±20.5[c]
Small thymus	0±0	0±0	16.7±16.7	0±0
Foramen ovale enlarged	0±0	1.5±1.5	3.3±3.3	0±0
Atrio-ventricular communis	0±0	3.0±3.0	0±0	0±0
Atrio-ventricular valve enlarged	0±0	1.5±1.5	0±0	0±0
Deflated lung	1.2±1.2	4.8±3.4	0±0	0±0
Kidney: hydronephrosis	1.2±1.2	0±0	0±0	0±0
small	0±0	0±0	3.3±3.3	0±0
cortex solidified	0±0	2.3±2.3	0±0	0±0
Distended urinary bladder	0±0	0±0	8.3±8.3	0±0

a/ Control group.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

c/ Significantly different from control (two sample rank test).

The skeletal anomalies observed in fetuses from mice exposed to 41 and 54 ppm of VDC are reported in Tables 14 and 15. An increased incidence of unossified incus occurred in the groups exposed to 41 ppm of VDC from gestational days 8 to 15 and 10 to 15. In addition, an increased incidence of unossified sternebrae occurred in the groups exposed from days 10 to 15. Ossification problems with the incus also occurred in mice exposed to 54 ppm of VDC from gestational days 8 to 15 and 12 to 15. Anomalies of the sternebrae occurred in the groups exposed to 54 ppm of VDC from gestational days 8 to 15 and 12 to 15. Incompletely ossified supraoccipitals occurred, at an increased frequency, in the groups exposed to VDC from gestational days 10 to 15 and 12 to 15. In addition, the normal ossification of centri was reduced in the group exposed from gestational days 8 to 15.

3. Mice exposed for 2- to 3-day intervals during gestation (Part 3):

a. General observations: Mice were exposed to 0, 56, 81 and 112 ppm of VDC for 2- to 3-day intervals beginning and ending at various times during gestation. In Part 3, one control group was used for all the treatment periods. However, each treatment period had its own feed restricted group. Mice in these groups were not given any feed during the appropriate intervals. At the end of the exposure period, all groups were given free access to feed.

The effects of a 3-day exposure, which started on gestational day 6, on maternal welfare and reproduction are presented in Table 16. A reduced ratio of pregnant to exposed mice occurred in all the VDC treated groups and the feed restricted group. Significant mortality occurred in the group exposed to 112 ppm of VDC and there were no pregnant survivors. Complete resorptions occurred in the one surviving dam from the group exposed to 81 ppm of VDC and the two surviving dams from the feed restricted group. Other than a significant reduction in the ratio of pregnant to exposed mice, there was no effect of 56 ppm VDC exposure on the parameters of maternal welfare and reproduction presented in Table 16.

The effects of a 3-day exposure, which started on gestational day 9, on maternal welfare and reproduction are presented in Table 17. A reduced ratio of pregnant to treated mice was observed in the group exposed to 56 ppm of VDC. Mortality occurred in the dams exposed to 112 ppm and there were no survivors. The weight gain during treatment was significantly reduced both in the group exposed to 81 ppm of VDC and the group starved during exposure. The weight gain of the latter group increased after exposure; however, this increase may reflect the greater weight loss during treatment. The group exposed to 81 ppm of VDC had a reduced percent viable fetuses, an increased percent of early resorptions, a reduced number of fetuses/dam, and a reduced fetal body weight. These effects did not reach a level of significance in the feed restricted group.

TABLE 14

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE AT 41 PPM ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

VDC (ppm)	0[a/]	41	41	0[b/]
Days Exposed	8 to 15	8 to 15	10 to 15	8 to 15
Number of				
Litters inspected	17	17	9	13
Fetuses insepcted	168	93	26	118
Skeletal anomalies				
Skull collapsed: slight	0±0[c/]	2.2±1.5	14.8±11.3	5.8±3.2
marked	0±0	0±0	0±0	7.3±4.3
Nasal bones: incompletely ossified	0±0	11.8±8.1	5.6±5.6	0±0
elevated	0±0	5.9±5.9	10.0±6.7	0±0
Occipital fontanel enlarged	1.0±1.0	17.8±8.5	0±0	0±0
Interparietals curved medially	0±0	1.0±1.0	5.9±4.1	0±0
Supraoccipital: unossified	2.4±2.3	15.3±8.5	0±0	0±0
incompletely ossified	4.3±2.6	25.3±6.6[d/]	8.3±5.9	3.8±3.8
Incus unossified	23.4±7.7	54.9±9.4[d/]	58.3±14.5[d/]	11.5±6.4
Hyoid bone: unossified	7.8±6.1	17.6±9.5	0±0	1.3±1.3
incompletely ossified	0±0	0±0	0±0	1.3±1.3
Sternebrae: ossified normally	61.4±7.8	54.0±8.4	42.0±12.5	67.6±7.2
unossified	7.8±6.1	19.7±9.4	26.9±11.1[d/]	0±0
incompletely ossified	7.1±3.3	14.7±6.8	29.8±11.7	2.6±1.7
split	6.4±3.0	12.2±4.0	28.1±11.4	13.1±7.1
fused	1.2±1.2	0±0	0±0	0±0
malaligned	14.4±4.4	14.9±4.3	3.7±3.7	21.9±5.1
Centri: ossified normally	100±0	99.0±1.0	100±0	100±0
Vertebrae: hemi-vertebra	0±0	1.0±1.0	0±0	0±0
Ribs: vertically or malfused	0±0	1.0±1.0	0±0	0±0
extra	22.8±6.5	13.1±4.9	40.6±14.1	33.8±8.4
wavy	0±0	1.0±1.0	0±0	1.9±1.9

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control (two sample rank test).

TABLE 15

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE AT 54 PPM ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

VDC (ppm)	0[a]	54	54	54[b]
Days Exposed	6 to 15	8 to 15	10 to 15	12 to 15
Number of				
Litters inspected	17	11	4	6
Fetuses inspected	168	55	15	17
Skeletal anomalies				
Skull collapsed: slight	0±0 [c]	3.6±3.6	0±0	0±0
Nasal bones: incompletely ossified	0±0	0±0	0±0	16.7±16.7
elevated	0±0	4.9±3.7	0±0	0±0
Occipital fontanel enlarged	1.0±1.0	11.4±6.2	33.3±23.6	61.1±20.0
Interparietals curved medially	0±0	4.9±3.7	0±0	0±0
Supraoccipital: unossified	2.4±2.3	0±0	0±0	18.1±12.6
incompletely ossified	4.3±2.6	21.7±9.0	57.5±21.7[d]	76.4±12.3[d]
Incus unossified	23.4±7.7	65.0±8.6[d]	95.0±5.0[d]	100±0[d]
Palatine bones incompletely ossified	0±0	0±0	0±0	16.7±16.7
Mandibles shortened	0±0	0±0	0±0	16.7±16.7
Hyoid bone: unossified	7.8±6.1	0±0	0±0	8.3±8.3
incompletely ossified	0±0	1.8±1.8	0±0	0±0
split	0±0	2.6±2.6	0±0	0±0
Sternebrae: ossified normally	61.4±7.8	38.1±7.0[d]	40.0±24.5	0±0[d]
unossified	7.8±6.1	7.3±7.3	41.7±25.0	88.9±7.2[d]
incompletely ossified	7.1±3.3	23.0±8.3	18.3±10.7	23.6±12.3
split	6.4±3.0	23.9±7.0[d]	37.5±23.9	25.0±12.0
fused	1.2±1.2	0±0	0±0	0±0
malaligned	14.4±4.4	30.6±5.7[d]	0±0	8.3±8.3
Centri: ossified normally	100±0	86.6±6.3[d]	100±0	100±0
fused vertically	0±0	4.3±2.2	0±0	0±0
malaligned	0±0	1.3±1.3	0±0	0±0
Vertebral: fused vertically	0±0	5.8±3.3	0±0	0±0
Ribs: vertically or malfixed	0±0	8.7±4.5	0±0	0±0
extra	22.8±6.5	25.3±8.2	45.0±16.6	11.1±7.0
wavy	0±0	0±0	25.0±25.0	0±0
Paws: incompletely ossified	1.2±1.2	4.4±4.4	0±0	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a litter basis.
d/ Significantly different from control (two sample rank test).

TABLE 16

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 9 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)				
	0[c]	56	81	112	0[d]
Number Exposed	12	16	16	14	17
Pregnant	10	5[e]	1[e]	6[e]	3[e]
Alive	10	5	1	0[e]	2
Non-pregnant	2	11	15	8	14
Alive	2	11	15	7	14
Body Weight Change[a]					
During exposure	2.1 ± 0.3	0.9 ± 0.8	-4.0	-	-7.5 ± 0.5[f]
After exposure	15.4 ± 0.7	20.0 ± 2.2	-2.2	-	4.2 ± 0.8[f]
Feed Consumption[b]					
During exposure	5.4 ± 0.2	1.7 ± 0.5	1.1 ± 0.2	-	0
After exposure	5.8 ± 0.4	5.2 ± 0.3	3.7 ± 0.6	-	5.3 ± 0.5
Pregnant Survivors	10	5	1	0	2
Implants/dam	10.7 ± 0.7	10.4 ± 0.9	12.0	-	6.5 ± 2.5
Viable fetuses (%)	91 ± 4	97 ± 3	0	-	0
Dead fetuses (%)	0	0	0	-	0
Early resorptions (%)	3 ± 1	2 ± 2	100[g]	-	100 ± 0[g]
Late resorptions (%)	5 ± 3	2 ± 2	0	-	0
Dams with complete resorptions	0	0	1	-	2
Live Litters	10	5	0	0	0
Fetuses/dam	9.6 ± 0.5	10.0 ± 0.8	-	-	-
Males (%)	53 ± 3	64 ± 6	-	-	-
Fetal weight (gm)	1.15 ± 0.04	1.12 ± 0.04	-	-	-

a/ Gm/animal/interval for pregnant mice.
b/ Gm/animal/day for pregnant mice.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (Tukey's omega procedure).
g/ Significantly different from control (two sample rank test).

TABLE 17

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 9 TO 12 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)				
	0[c]	56	81	112	0[d]
Number Exposed	12	16	19	12	14
Pregnant	10	6[e]	12	7	7
Alive	10	5	12	0[e]	7
Non-pregnant	2	10	7	5	7
Alive	2	10	7	4	7
Body Weight Change[a]					
During exposure	3.9 ± 0.3	-0.4 ± 2.2	-4.6 ± 1.0[f]	-	-8.0 ± 0.7[f]
After exposure	11.5 ± 0.6	19.6 ± 1.5[g]	14.8 ± 1.2	-	19.5 ± 1.4[g]
Feed Consumption[b]					
During exposure	5.2 ± 0.4	1.9 ± 0.2	1.5 ± 0.2	-	0
After exposure	6.4 ± 0.2	5.0 ± 0.2	4.6 ± 0.3	-	7.0 ± 2.5
Pregnant Survivors	10	5	12	0	7
Implants/dam	10.7 ± 0.7	11.2 ± 0.4	11.2 ± 0.4	-	11.6 ± 0.8
Viable fetuses (%)	91 ± 4	93 ± 3	65 ± 7[f]	-	78 ± 11
Dead fetuses (%)	0	2.0 ± 2.0	0	-	0
Early resorptions (%)	3 ± 1	2 ± 2	22 ± 8[f]	-	4 ± 3
Late resorptions (%)	5 ± 3	4 ± 4	14 ± 3	-	19 ± 9
Dams with complete resorptions	0	0	1	-	0
Live Litters	10	5	11	0	7
Fetuses/dam	9.6 ± 0.5	10.4 ± 0.6	7.8 ± 0.4[f]	-	9.1 ± 1.6
Males (%)	53 ± 3	41 ± 2	54 ± 3	-	54 ± 6
Fetal weight (gm)	1.15 ± 0.04	1.16 ± 0.07	0.88 ± 0.04[g]	-	1.01 ± 0.04

a/ Gm/animal/interval for pregnant mice.
b/ Gm/animal/day for pregnant mice.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (two sample rank test).
g/ Significantly different from control (Tukey's omega procedure).

The effects of a 3-day exposure, which started on gestational day 12, on maternal welfare and reproduction are presented in Table 18. A reduced ratio of pregnant to exposed mice occurred in the group treated with 81 ppm of VDC. Deaths occurred in the dams exposed to both 81 and 112 ppm of VDC. The weight gain during treatment was reduced in the groups exposed to 81 and 112 ppm VDC and the feed restricted group. The weight gain after treatment was normal for all of the groups except the group exposed to 112 ppm of VDC. A significant increase in early resorptions occurred in all of the VDC exposed groups and the feed restricted group. In addition, there was a significant increase in the ratio of dams with complete resorptions to pregnant survivors in the 112 ppm VDC exposure group and the feed restricted group. The number of fetuses/dam and the fetal body weight was reduced only in the feed restricted group.

The effect of a 2-day exposure, which started on gestational day 15, on maternal welfare and reproduction is presented in Table 19. A significant reduced ratio of pregnant to exposed mice occurred in the 81 ppm VDC treated group. There was no significant increase in mortality in any of the groups. The weight gain during treatment was reduced for all of the VDC exposed groups and the feed restricted group. After exposure, the weight gain after treatment was normal for both the 56 and 81 ppm VDC exposed groups. The percent viable fetuses was reduced for all of the VDC exposed groups and the feed restricted group. None of the dams in the groups exposed to 112 ppm of VDC produced any viable fetuses. At the time of examination, the implants in these dams were classified as being a mixture of early and late resorptions. Increased resorptions also occurred in the feed restricted group. Fetal body weights were reduced in the 56 and 81 ppm exposed groups as well as the feed restricted group.

b. Anomalies: Gross anomalies observed in fetuses from dams exposed to 56 and 81 ppm of VDC are presented in Table 20. Immature skin and hematoma were the most frequent anomalies observed. The incidence of these anomalies was increased on the group exposed to 81 ppm of VDC from gestational days 15 to 17. In addition, this group also had an increased incidence of runting among the fetuses. Hematomas were also observed in the feed restricted group.

Soft tissue anomalies observed in fetal mice from dams exposed to various concentrations of VDC for 2- to 3-day intervals during gestation are presented in Tables 21 to 24. A significant increase in hydrocephalus of the lateral ventricles occurred in mice from the groups exposed to 56 and 81 ppm of VDC from gestational days 15 to 17 (Table 24). However, this anomaly occurred at a similar, although not statistically significant, frequency in the feed restricted group. Small kidneys were more frequent in mice from the group exposed to 81 ppm of VDC from gestational days 15 to 17. Although some soft tissue anomalies were observed in all of the groups, none of these anomalies, other than those described above, occurred at a significantly greater frequency in the VDC-treated groups relative to the control groups.

TABLE 18

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 12 TO 15 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)				
	0[c]	56	81	112	0[d]
Number Exposed	12	7	24	8	15
Pregnant	10	5	6[e]	6	10
Alive	10	5	2[e]	3[e]	10
Non-pregnant	2	2	20	2	5
Alive	2	2	20	0	5
Body Weight Change[a]					
During exposure	4.9 ± 0.5	0.4 ± 2.0	-6.0 ± 3.0[f]	-9.0 ± 2.0[f]	-6.3 ± 0.4[f]
After exposure	6.6 ± 0.6	6.2 ± 1.3	6.3 ± 2.8	-2.3 ± 0.6[g]	7.7 ± 1.3
Feed Consumption[b]					
During exposure	5.6 ± 0.4	1.7 ± 0.5	1.7 ± 0.1	1.0 ± 0.0	0
After exposure	6.7 ± 0.6	4.5 ± 0.5	7.7 ± 1.8	1.2 ± 0.0	6.1 ± 0.6
Pregnant Survivors	10	5	2	3	10
Implants/dam	10.7 ± 0.7	8.2 ± 2.3	8.5 ± 3.5	6.7 ± 2.7	10.6 ± 0.9
Viable fetuses (%)	91 ± 4	71 ± 19	42 ± 42	0	22 ± 9[f]
Dead fetuses (%)	0	0	0	0	0
Early resorptions (%)	3 ± 1	22 ± 20[f]	58 ± 42[f]	100 ± 0[f]	55 ± 12[f]
Late resorptions (%)	5 ± 3	8 ± 8	0	0	23 ± 10
Dams with complete resorptions	0	1	1	3[e]	4[e]
Live Litters	10	4	1	0	6
Fetuses/dam	9.6 ± 0.5	6.3 ± 2.1	10	-	4.2 ± 1.4[g]
Males (%)	53 ± 3	61 ± 13	50	-	51 ± 14
Fetal weight (gm)	1.15 ± 0.04	1.17 ± 0.16	0.83	-	0.86 ± 0.06[g]

a/ Gm/animal/interval for pregnant mice.
b/ Gm/animal/day for pregnant mice.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (two sample rank test).
g/ Significantly different from control (Tukey's omega procedure).

TABLE 19

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 15 TO 17 ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Vinylidene Chloride (ppm)				
	0[c]	56	81	112	0[d]
Number Exposed	12	8	24	7	16
Pregnant	10	7	11[e]	6	9
Alive	10	7	11	4	9
Non-pregnant	2	1	13	1	7
Alive	2	1	13	0	7
Body Weight Change[a]					
During exposure	6.2 ± 0.7	-5.1 ± 0.3[f]	-5.5 ± 0.7[f]	-4.0 ± 1.1[f]	-9.3 ± 0.4[f]
After exposure	0.4 ± 0.7	0.3 ± 0.7	0.7 ± 0.9	-5.5 ± 2.1[g]	6.6 ± 0.5[g]
Feed Consumption[b]					
During exposure	5.4 ± 0.6	2.2 ± 0.4	1.8 ± 0.2	0.3 ± 0.2	0
After exposure	5.5 ± 2.0	3.3 ± 1.2	2.4 ± 0.5	1.2 ± 0.5	5.2 ± 2.7
Pregnant Survivors	10	7	11	4	9
Implants/dam	10.7 ± 0.7	11.0 ± 0.6	12.5 ± 0.3	12.3 ± 0.8	10.7 ± 0.7
Viable fetuses (%)	91 ± 4	69 ± 8[f]	47 ± 12[f]	0[f]	48 ± 14[f]
Dead fetuses (%)	0	0	0	0	0
Early resorptions (%)	3 ± 1	17 ± 7	29 ± 12	75 ± 22[f]	38 ± 12[f]
Late resorptions (%)	5 ± 3	14 ± 7	24 ± 9	25 ± 22	14 ± 8
Dams with complete resorptions	0	0	3	4[e]	3
Liver Litters	10	7	8	0	6
Fetuses/dam	9.6 ± 0.5	7.7 ± 1.1	8.3 ± 1.4	-	7.0 ± 1.1
Males (%)	53 ± 3	55 ± 6	54 ± 9	-	38 ± 5
Fetal weight (gm)	1.15 ± 0.04	0.66 ± 0.03[g]	0.55 ± 0.02[g]	-	0.81 ± 0.03[g]

a/ Gm/animal/interval for pregnant mice.
b/ Gm/animal/day for pregnant mice.
c/ Control group.
d/ Feed restricted group.
e/ Significantly different from control (Fisher's exact probability test).
f/ Significantly different from control (two sample rank test).
g/ Significantly different from control (Tukey's omega procedure).

TABLE 20

EFFECT OF VINYLIDENE CHLORIDE (VDC) EXPOSURE ON THE INCIDENCE OF GROSS ANOMALIES IN MICE

VDC (ppm)	Gestational Days Exposed	Gross Anomalies: Immature Skin	Hematoma	Runting
0[a/]	6-17	3.3±2.4[c/]	0.8±0.8	0±0
56	6-9	0±0	4.0±4.0	0±0
	9-12	4.4±4.4	5.5±5.5	0±0
	12-15	2.3±2.3	0±0	0±0
	15-17	0±0	1.3±1.3	0±0
81	9-12	18.7±9.5	5.6±2.6	0±0
	12-15	0	0	0
	15-17	18.7±6.0[d/]	9.0±3.4[d/]	71.4±18.4[d/]
0[b/]	9-12	0±0	6.1±4.7	0±0
	12-15	5.5±5.5	33.7±15.1[d/]	0±0
	15-17	0±0	10.5±4.3[d/]	0±0

a/ Control group.

b/ Feed restricted group.

c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

d/ Significantly different from control (two sample rank test).

TABLE 21

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 9 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Vinylidene Chloride (ppm)	
	0 [a]	56
Number of		
Litters inspected	10	5
Fetuses inspected	46	24
Soft tissue anomalies		
Hydrocephalus: lateral ventricle	0±0 [b]	5.0±5.0
fourth ventricle	5.0±3.3	4.0±4.0
Nasopharyngeal canal occluded	1.7±1.7	0±0
Nasal passage occluded	7.3±4.9	25.3±12.7
Deflated lung	2.5±2.5	0±0
Kidney: hydronephrosis	1.7±1.7	0±0
small	0±0	4.0±4.0
cortex solidified	0±0	3.3±3.3

a/ Control group.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

TABLE 22

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 9 TO 12 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0 [a]	56	81	0 [b]
Number of				
Litters inspected	10	5	11	7
Fetuses inspected	46	24	40	29
Soft tissue anomalies				
Hydrocephalus: third ventricle	0±0 [c]	0±0	3.0±3.0	0±0
fourth ventricle	5.0±3.3	0±0	6.8±4.9	2.9±2.9
Nasopharyngeal canal occluded	1.7±1.7	0±0	0±0	0±0
Nasal passage occluded	7.3±4.9	0±0	10.6±6.4	7.1±4.6
Micropthalmia	0±0	0±0	2.3±2.3	0±0
Ectopic eye	0±0	0±0	2.3±2.3	0±0
Cleft palate	0±0	0±0	9.1±9.1	25.0±14.4
Deflated lung	2.5±2.5	0±0	9.8±4.2	14.3±14.3
Kidney: hydronephrosis	1.7±1.7	4.0±4.0	0±0	0±0
small	0±0	0±0	4.5±4.5	0±0
cortex solidified	0±0	0±0	3.0±3.0	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a litter basis.

TABLE 23

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 12 TO 15 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0 [a]	56	81	0 [b]
Number of				
Litters inspected	10	4	1	6
Fetuses inspected	46	12	5	13
Soft tissue anomalies				
Hydrocephalus: lateral ventricle	0±0 [c]	0±0	60	25.0±15.8
fourth ventricle	5.0±3.3	0±0	0	0±0
Nasopharyngeal canal occluded	1.7±1.7	12.5±12.5	0	0±0
Nasal passage occluded	7.3±4.9	0±0	20	12.5±8.5
Cleft palate	0±0	16.3±9.9	20	25.0±14.4
Deflated lung	2.5±2.5	0±0	20	4.2±4.2
Kidney: hydronephrosis	1.7±1.7	0±0	0	8.3±8.3
small	0±0	0±0	0	4.2±4.2
cortex solidified	0±0	0±0	20	4.2±4.2
Distended urinary bladder	0±0	25.0±25.0	0	0±0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. or mean of the percent of fetuses with the indicated anomaly calculated on a litter basis.

TABLE 24

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 15 TO 17 ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0 [a]	56	81	0 [b]
Number of				
Litters inspected	10	7	8	6
Fetuses inspected	46	25	34	20
Soft tissue anomalies				
Hydrocephalus: lateral ventricle	0±0 [c]	27.1±13.5 [d]	26.3±11.8 [d]	27.8±12.7
third ventricle	0±0	0±0	11.3±5.9	15.8±8.2
fourth ventricle	5.0±3.3	0±0	13.3±5.3	0±0
Subarachnoidal space enlarged	0±0	0±0	5.0±5.0	0±0
Nasopharyngeal canal occluded	1.7±1.7	0±0	0±0	0±0
Nasal passage occluded	7.3±4.9	4.8±4.8	10.4±8.3	0±0
Micropthalmia	0±0	0±0	2.5±2.5	0±0
Cleft palate	0±0	0±0	20.8±12.5	8.3±8.3
Deflated lung	2.5±2.5	2.9±2.9	8.3±6.3	4.2±4.2
Ductus venosis hemorrhage	0±0	7.1±7.1	0±0	0±0
Kidney: hydronephrosis	1.7±1.7	0±0	0±0	0±0
small	0±0	10.0±7.2	20.8±8.2 [d]	7.5±4.8
cortex solidified	0±0	2.8±2.8	0±0	0±0
Distended urinary bladder	0±0	7.1±7.1	0±0	0±0
Misplaced testes	0±0	0±0	2.1±2.1	0±0
Misplaced ovary	0±0	0±0	2.1±2.1	0±0

[a] Control group.
[b] Feed restricted group.
[c] Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a litter basis.
[d] Significantly different from control group (two sample rank test).

Skeletal anomalies observed in fetal mice from dams exposed to various concentrations of VDC for 2- to 3-day intervals during gestation are presented in Tables 25 to 28. No increase in skeletal anomalies was produced in the group of mice exposed to 56 ppm of VDC from gestational days 6 to 9 (Table 25). In addition, no increase in skeletal anomalies was observed when mice were exposed to this concentraton of VDC from gestational days 9 to 12 (Table 26) or 12 to 15 (Table 27). However, if mice were exposed to 56 ppm of VDC from gestational days 15 to 17 then there was an increase in unossified supraoccipitals, hyoid bones, and sternabrae (Table 28). In addition, there was a reduction in the number of normally ossified sternabrae and a corresponding reduction in the incidence of malaligned sternabrae. If the concentration of VDC was increased to 81 ppm then anomalies of the sternebrae and centri were observed in the group exposed from gestational days 9 to 12 (Table 26). However, these anomalies also occurred at a similar frequency in the feed restricted group. There were not enough litters in the group exposed to 81 ppm of VDC from gestational days 12 to 15 to determine if exposure during this period produced skeletal anomalies. If mice were exposed to 81 ppm of VDC from gestational days 15 to 17 then there was an increased incidence of both slight and marked collapsed skull (Table 28). This type of anomaly did not occur in the feed restricted group. In addition, there were also unossified supraoccipitals, sternabrae, and paws as well as malaligned sternabrae in the group exposed to 81 ppm of VDC.

C. Behavioral Studies on Rats

1. General observations: General observations, which summarize the effects of VDC exposure on maternal welfare and reproduction, are presented in Table 29. No deaths occurred during exposure. A weight loss occurred during the initial portion of the exposure period in the VDC exposed groups as well as the feed restricted group. During the latter portion of the exposure period, only the high dose VDC group lost weight. The total weight gain during the exposure period was reduced in both VDC exposed groups and the feed restricted group. The body weight of day old pups in the normalized litters was also reduced in these groups. There was a trend towards a reduced number of viable pups in the high dose VDC group. In conjunction with this observation, there was a significant reduction in the number of pups/implants in this group. The number of implants were determined at weaning when the dam was sacrificed and the uterus examined for implantation sites.

2. Behavioral observations: Although there were significant effects due to sex on some of the tests, in no case was there a significant sex times treatment interaction. Since we are only interested in effects due to treatments, and these effects were not differentially affected by sex, the data from both sexes were combined for presentation.

TABLE 25

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 6 TO 9 ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Vinylidene Chloride (ppm)	
	0[a/]	56
Number of		
Litters inspected	10	5
Fetuses inspected	50	26
Skeletal anomalies		
Skull collapsed: slight	4.0±2.7[b/]	5.0±5.0
Nasal bones: curved, medially	7.7±6.0	11.4±11.4
Occipital fontanel enlarged	4.0±2.7	16.0±11.7
Supraoccipital: unossified	2.0±2.0	0±0
incompletely ossified	9.3±5.0	17.0±7.7
Incus unossified	28.0±9.3	26.7±14.6
Sternebrae: ossified normally	44.3±8.2	40.1±16.9
unossified	10.0±8.0	17.0±11.8
incompletely ossified	26.3±5.1	28.0±13.6
split	9.3±4.0	0±0
malaligned	25.3±5.9	18.9±9.0
extra	2.0±2.0	0±0
Centri: ossified normally	100±0	100±0
Paws: unossified	4.0±2.7	0±0
phalanges unossified	13.3±6.4	29.0±10.0

a/ Control group.

b/ Mean ± SE of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

TABLE 26

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 9 TO 12 ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0[a]	56	81	0[b]
Number of				
Litters inspected	10	5	11	7
Fetuses inspected	50	28	47	34
Skeletal anomalies				
Skull collapsed: slight	4.0±2.7[c]	8.0±4.9	7.1±3.8	2.9±2.9
marked	0±0	0±0	6.8±4.9	0±0
Nasal bones: curved medially	7.7±6.0	14.7±9.0	14.1±6.1	11.3±9.4
Occipital fontanel enlarged	4.0±2.7	4.0±4.0	2.3±2.3	0±0
Supraoccipital: unossified	2.0±2.0	0±0	2.3±2.3	0±0
incompletely ossified	9.3±5.0	17.3±9.2	6.4±4.2	24.2±8.2
Incus unossified	28.0±9.3	13.3±9.7	47.3±13.6	52.9±17.8
Hyoid bone unossified	0±0	0±0	6.8±6.8	0±0
Sternebrae: ossified normally	44.3±8.2	63.3±17.0	25.6±6.8[d]	29.6±13.0
unossified	10.0±8.0	4.0±4.0	22.9±7.1	9.9±3.6
incompletely ossified	26.3±5.1	18.7±12.2	26.7±8.0	27.3±13.6
lobed	0±0	0±0	0±0	1.8±1.8
split	9.3±4.0	10.0±6.7	54.1±10.4[d]	40.8±13.3[d]
maligned	25.3±5.9	7.3±4.5	17.1±4.4	21.1±6.3
extra	2.0±2.0	3.3±3.3	0±0	0±0
Centri: ossified normally	100±0	100±0	76.1±7.4[d]	95.2±4.8
lobed	0±0	0±0	16.7±7.9	4.8±4.8
incompletely ossified	0±0	0±0	5.5±2.8	0±0
Paws: unossified	4.0±2.7	0±0	0±0	0±0
phalanges unossified	13.3±6.4	8.0±8.0	62.3±11.1	21.1±8.2

a/ Control group.
b/ Feed restricted group.
c/ Mean ± SE of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control (two sample rank test).

TABLE 27

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 12 TO 15 ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0[a]	56	81	0[b]
Number of				
Litters inspected	10	3	1	4
Fetuses inspected	50	13	5	12
Skeletal anomalies				
Skull collapsed: slight	4.0±2.7[c]	16.7±16.7	80	25.0±25.0
Nasal bones: curved medially	7.7±6.0	0±0	80	0±0
Occipital fontanel enlarged	4.0±2.7	0±0	0	0±0
Supraoccipital: unossified	2.0±2.0	11.1±11.1	0	5.0±5.0
incompletely ossified	9.3±5.0	50.0±9.6	100	56.3±21.3
Incus unossified	28.0±9.3	22.2±22.2	100	25.0±25.0
Sternebrae: ossified normally	44.3±8.2	30.6±19.4	0	5.0±5.0[d]
unossified	10.0±8.0	41.7±30.0	100	67.5±19.7[d]
incompletely ossified	26.3±5.1	30.6±2.8	40	27.5±16.0
split	9.3±4.0	33.3±33.3	100	47.5±20.6[d]
malaligned	25.3±5.9	8.3±8.3	20	23.8±10.3
extra	2.0±2.0	0±0	0	0±0
Centri: ossified normally	100±0	100±0	100	100±0
Paws: unossified	4.0±2.7	5.6±5.6	0	0±0
phalanges unossified	13.3±6.4	5.6±5.6	100	61.3±17.1

a/ Control group.
b/ Feed restricted group.
c/ Mean ± SE or individual value of the percent of fetuses with the indicated anomaly calculated on a per litter basis.
d/ Significantly different from control (two sample rank test).

TABLE 28

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 15 TO 17 ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Vinylidene Chloride (ppm)			
	0[a]	56	81	0[b]
Number of				
Litters inspected	10	7	7	6
Fetuses inspected	50	29	32	22
Skeletal anomalies				
Skull collapsed: slight	4.0±2.7[c]	12.4±9.5	40.0±12.6[d]	0±0
marked	0±0	0±0	21.9±10.1[d]	0±0
Nasal bones: incompletely ossified	0±0	14.3±9.2	31.0±18.0	25.0±17.1
curved, medially	7.7±6.0	29.3±11.3	0±0	9.7±6.2
Occipital fontanel enlarged	4.0±2.7	7.1±7.1	25.2±13.6	29.2±16.4
Supraoccipital: unossified	2.0±2.0	39.3±17.1[d]	52.9±14.6[d]	33.3±21.1
incompletely ossified	9.3±5.0	56.0±17.7	24.8±9.4	30.6±10.0
Incus unossified	28.0±9.3	90.0±7.2	64.3±18.0	80.6±16.3
Hyoid bone unossified	0±0	75.7±14.5[d]	40.5±19.2	6.9±4.5
Sternebrae: ossified normally	44.3±8.2	0±0[d]	0±0[d]	0±0[d]
unossified	10.0±8.0	91.9±5.8[d]	100±0[d]	93.1±4.5[d]
incompletely ossified	26.3±5.1	38.1±14.4	16.7±14.1	47.2±12.7
split	9.3±4.0	30.7±13.2	4.8±4.8	58.3±14.0[d]
malaligned	25.3±5.9	5.7±5.7[d]	0±0[d]	6.9±4.5[d]
extra	2.0±2.0	0±0	0±0	0±0
Centri: ossified normally	100±0	100±0	97.1±2	100±0
unossified	0±0	0±0	2.9±2.9	0±0
Pelvis: incompletely ossified	0±0	0±0	2.9±2.9	0±0
Paws: unossified	4.0±2.7	14.3±9.2	50.0±11.7[d]	0±0
incompletely ossified	0±0	0±0	11.9±7.9	25.0±17.1
phalanges unossified	13.3±6.4	92.9±7.1	45.2±13.6	45.8±15.0

a/ Control group.

b/ Feed restricted group.

c/ Mean ± SE of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

d/ Significantly different from control (two sample rank test).

TABLE 29

EFFECT OF VINYLIDENE CHLORIDE EXPOSURE ON GESTATIONAL DAYS 8 TO 20 ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Vinylidene Chloride (ppm)			
	0[b]	56	283	0[c]
Number Exposed	24	20	19	17
Pregnant	17	18	16	17
Alive	17	18	16	17
Non-pregnant	7	2	3	0
Alive	7	2	3	0
Body Weight Change[a]				
Days 8 to 13	8±8	-60±4[d]	-80±4[d]	-54±2[d]
Days 13 to 20	68±9	53±9	32±10[d]	54±2
Days 8 to 20	76±12	-7±8[d]	-45±10[d]	-0.6±4[d]
Pregnant Survivors	17	18	16	17
Implants/dam	13.3±0.4	12.4±0.8	11.8±1.0	12.8±0.7
Dams with complete resorptions	0	0	3	0
Live Litters	17	18	13	17
Pups/dam				
Actual	12.4±0.5	11.7±0.8	9.5±1.1	11.6±0.6
Normalized	8	8	8	8
Pup weight (gm)	7.0±0.2	5.7±0.2[d]	5.5±0.2[d]	6.3±0.2[d]
Pups/implants x 100	93±2	94±2	78±6[e]	92±2

a/ Gm/animal/interval for pregnant rats.
b/ Control group.
c/ Feed restricted group.
d/ Significantly different from control (Tukey's omega procedure).
e/ Significantly different from control (two sample rank test).

a. Preweaning tests:

(1) Surface righting: All of the groups were significantly different from the control group with respect to the age at which the pups were able to right themselves in 1 sec or less (Figure 2). The feed restricted group consistently mastered the task sooner than the controls did, and the two treatment groups were slower. However, the low and high dose groups could not be distinguished from each other.

(2) Pivoting: Although the feed restricted group spent the least time pivoting and the high dose group and low dose group, respectively, spent the most time pivoting, these differences did not reach statistical significance (Figure 3).

(3) Auditory startle: There were no significant effects due to treatment. The females did show the startle response sooner than males, but this was true across all treatments (Figure 4).

(4) Bar holding: Although the high dose group did reach the criterion of hanging on the bar for 15 sec sooner than the other groups the difference did not reach statistical significance (Figure 5). However, when the data were analyzed for each group as the total time spent hanging on the bar, then the high dose group did significantly better ($p < 0.001$). In other words, the high dose group did hang on the bar longer than the other three groups, but they did not reach the 15 sec criterion significantly sooner.

(5) Righting in air: In this test, the feed restricted and low dose groups took significantly longer to show the response, while the high dose group was not distinguishable from the control group (Figure 6).

(6) Visual placing: This test proved to be a difficult one to perform. The rat had to approach the ledge very close to elicit a response and then it was difficult to be sure that vibrissal contact had not occurred. It was also nearly impossible to standardize this test. Because of these problems the data were not analyzed.

(7) Swimming ability: There were no significant effects due to sex or treatment in the swimming test (Figure 7). The feed restricted and high dose groups did reach the score of 2 a little sooner than the other two groups, but the difference was only marginally significant ($p < 0.06$).

(8) Physical maturation: The high dose group showed detachment of the external ear earlier and eruption of the teeth significantly later than the controls ($p < 0.05$ and 0.01, respectively) (Figure 8). For the teeth, the feed restricted and low dose groups also showed later maturation relative to the controls. There was a significant sex effect for time of eye opening, with the females maturing earlier. However, this was true across treatment groups.

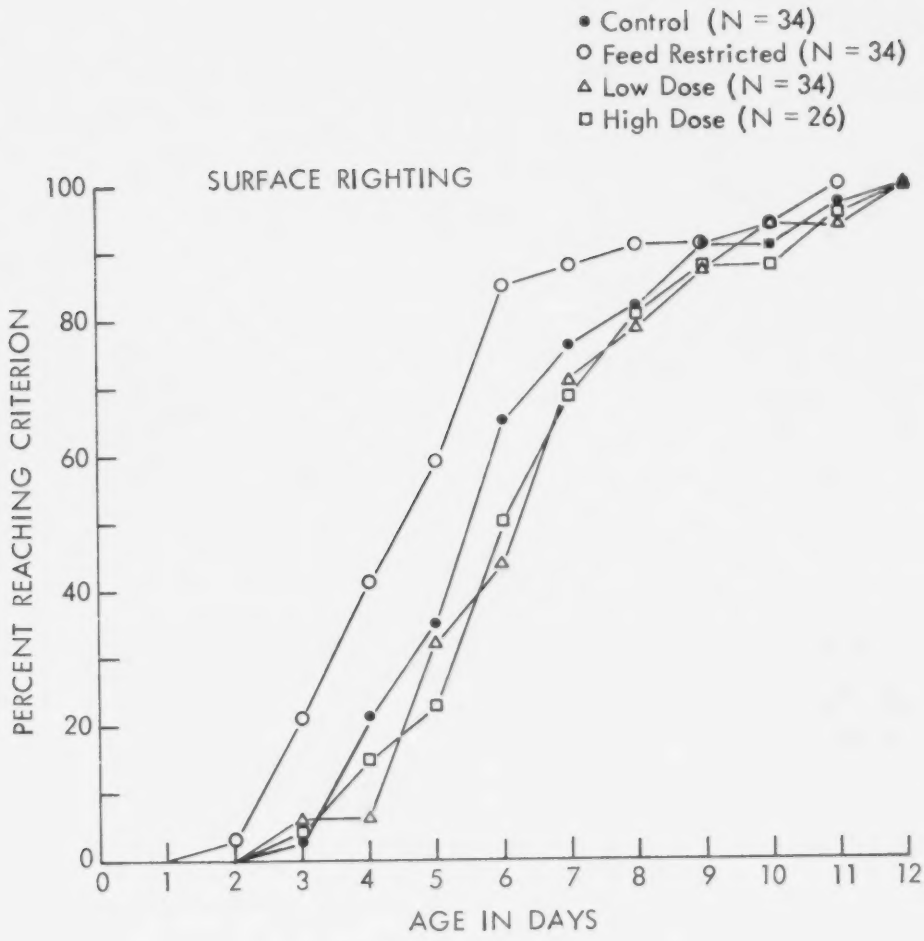

Figure 2 - Cumulative Percent of Rats, Sexes Combined, Reaching a Criterion of 1 Sec or Less for Righting on a Flat Surface. All groups were significantly different from controls at the 0.01 level.

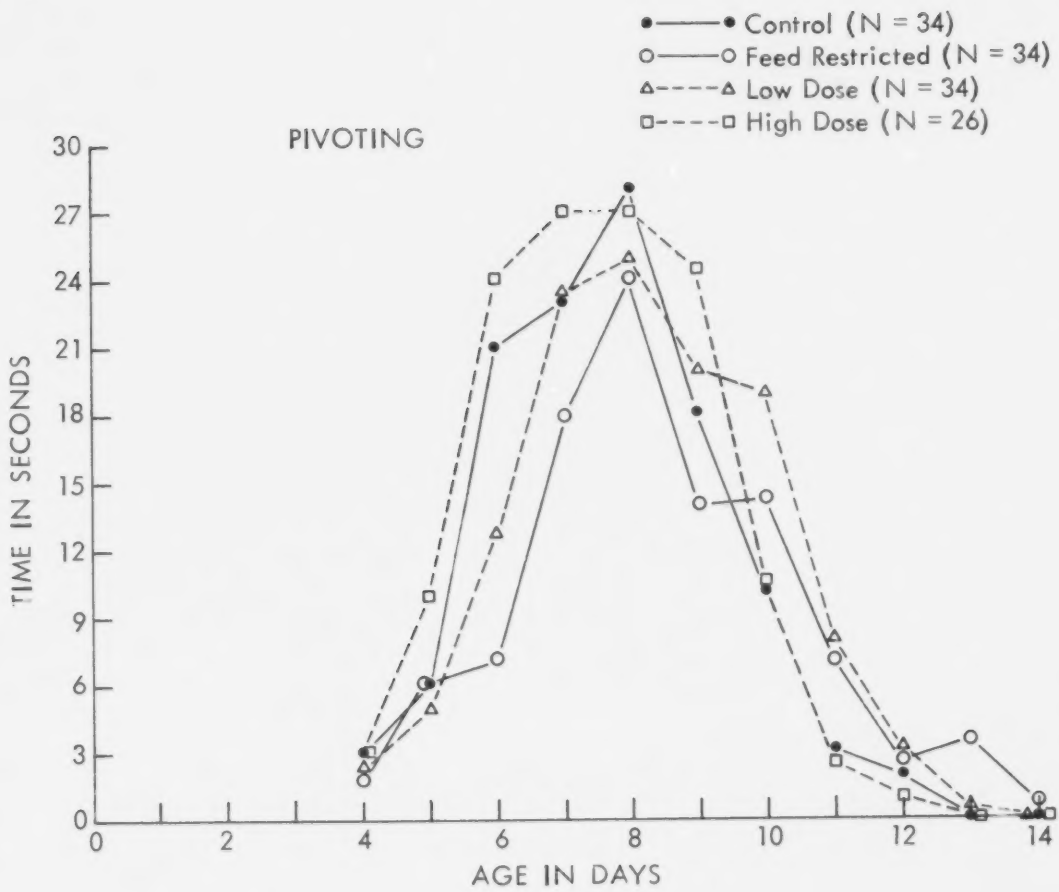

Figure 3 - Amount of Time Spent Pivoting During a 3-Min Test Period, Sexes Combined.

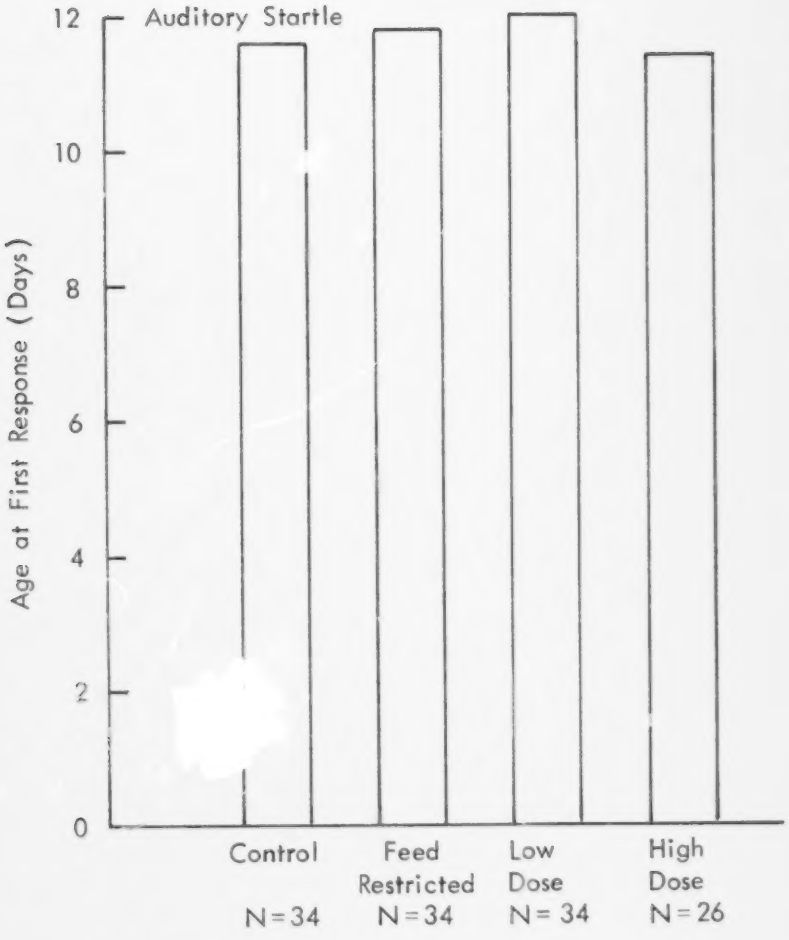

Figure 4 - First a Complete Startle Response Was Observed for Various Groups of Rats, Sexes Combined.

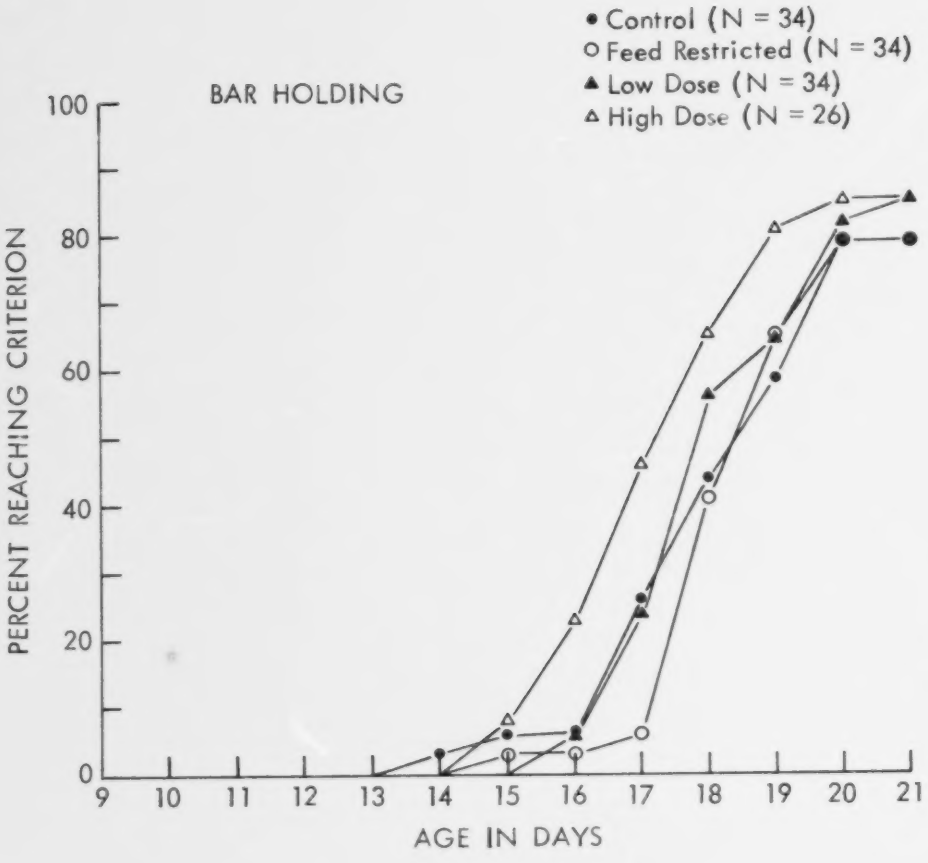

Figure 5 - Cumulative Percent of Rats, Sexes Combined, Reaching a Criterion of 15 Sec on the Bar-Holding Apparatus. These differences are not significant, but the high dose group did spend significantly ($p < 0.001$) more time hanging on the bar (see text).

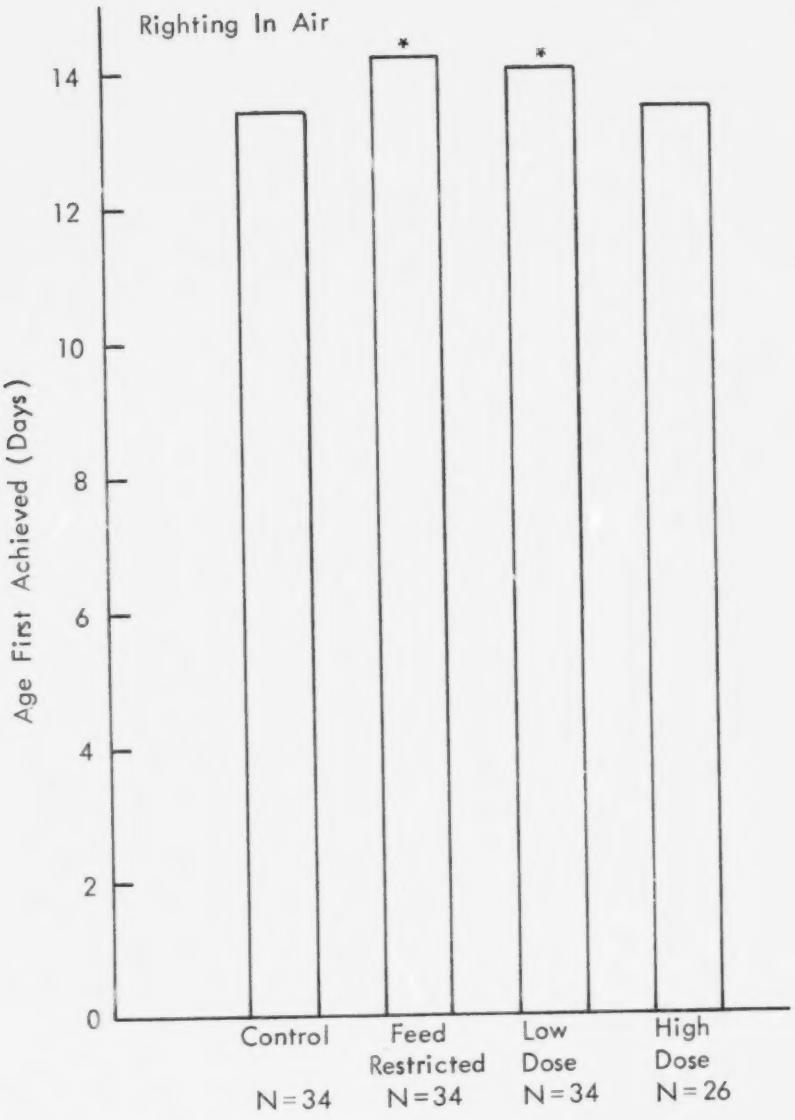

Figure 6 - First Day of Appearance for Righting in Air for Groups of Rats, Sexes Combined. * = statistically significant from the control group at the 0.05 level.

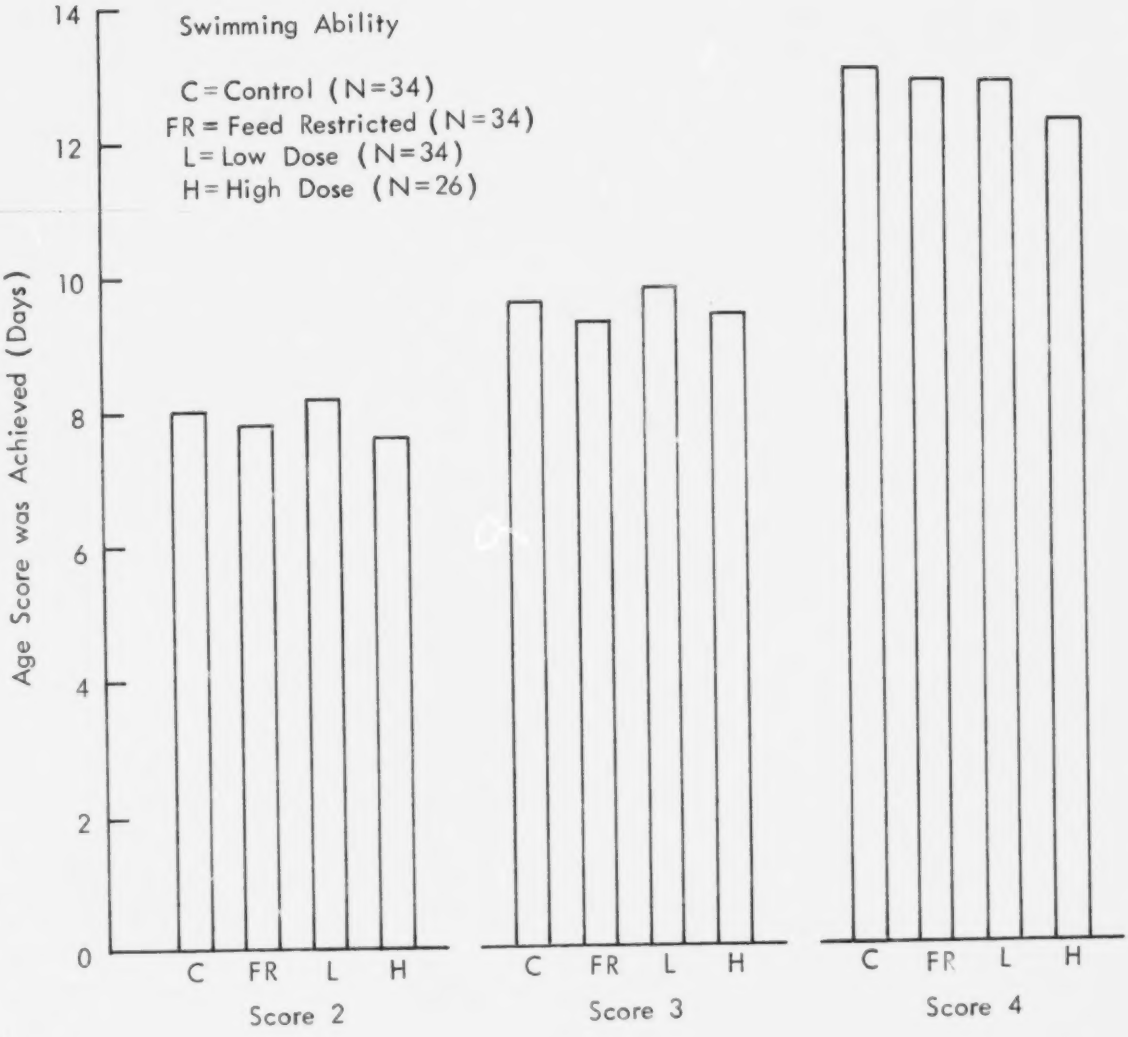

Figure 7 - First Day for Score of 2, 3, or 4 in the Swimming Test, Sexes Combined. The difference for pair-fed and high dose in reaching the score 2 was marginally significant ($p < 0.06$).

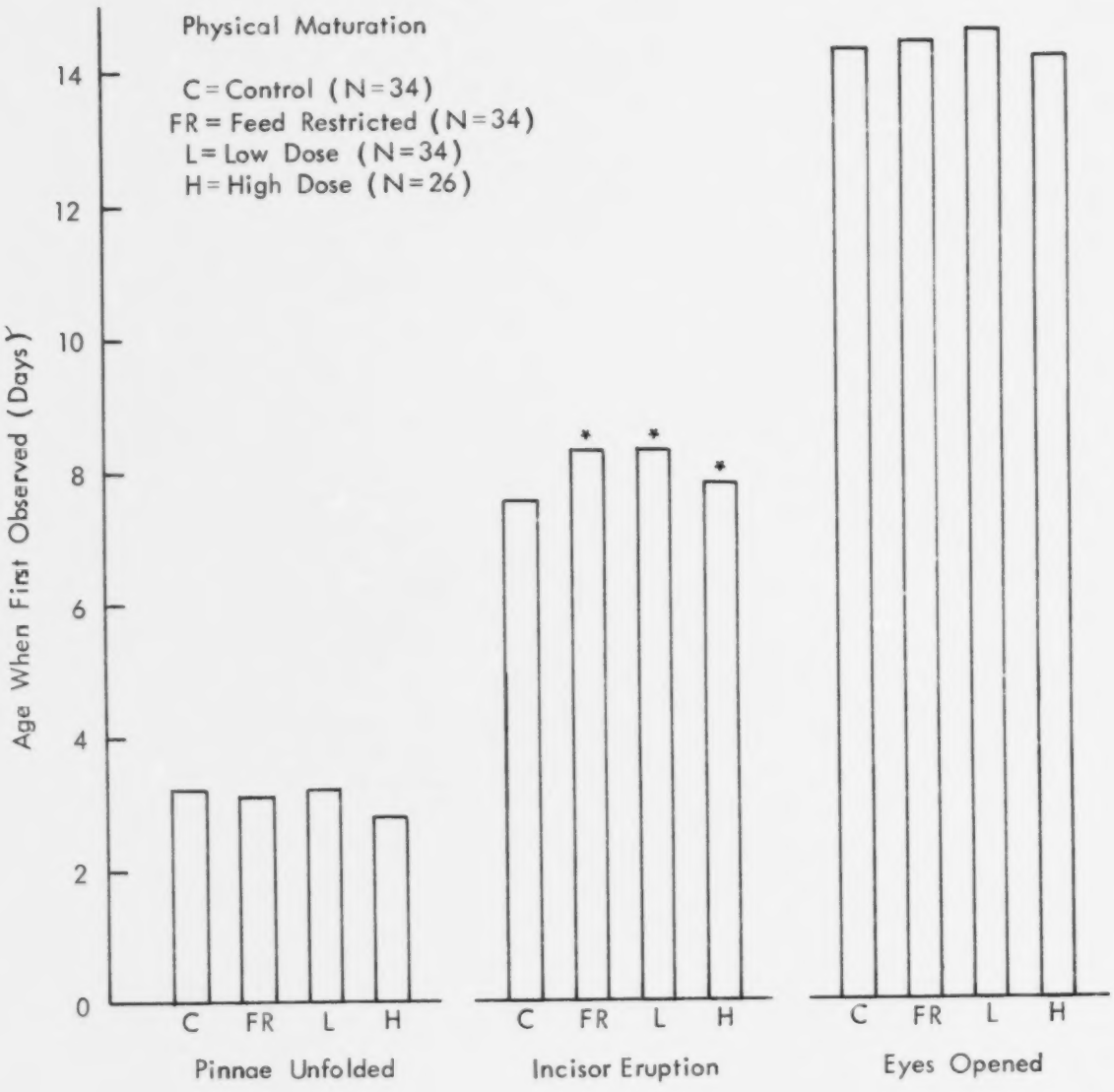

Figure 8 - First Day of Appearance of Physical Parameters for Groups of Rats, Sexes Combined. * = statistically significant from the control group at the 0.05 (pinnae) and 0.01 (incisor) levels.

There were highly significant differences in the body weights (Figure 9). On the average, the feed restricted group was lower than the control group, with the low dose and high dose groups lower still. These differences decreased with time and disappeared by 3 weeks of age (average weight: control, 52 g; feed restricted, 53 g; low dose, 52 g; high dose, 52 g).

b. Activity test: The only significance found in the activity data was an overall higher level of activity for the female rats (Figure 10). There was no interaction of sex with treatment, however. As expected for a nocturnal animal, the rats were more active during the dark.

Even though the rats were placed into the mazes during the light phase of their cycle, the first few hours produced the greatest counts of all. This is generally called exploratory behavior. We looked at exploratory behavior separately in the analysis by considering the first 2 hr apart from the general analysis. Here again, however, there were no significant differences attributable to the treatments.

IV. DISCUSSION

Pregnant mice and rats were exposed for approximately 23 hr a day to various concentrations of vinylidene chloride. The exposure periods included both organogenesis and portions of organogenesis. The purpose of this study was to determine if VDC altered normal development. Development was monitored in terms of the morphological and behavioral parameters of offspring from both control and VDC exposed animals. In addition, aspects of maternal welfare were also monitored in order to evaluate the toxicity of VDC in the dam.

A. Morphological Study

1. Maternal welfare: VDC was more toxic to adult mice than adult rats. No pregnant mice survived in atmospheres that contained either 144 or 300 ppm of VDC (Table 2). In contrast, more than half of the pregnant rats survived exposure to 300 and 449 ppm of VDC (Table 3). Maternal welfare, as measured in terms of weight change and feed consumption, was affected by exposure to VDC. The weight gain of rats was reduced during a 10 day exposure to 15 ppm of VDC (Table 3). At the end of exposure, these dams gained more weight than controls. Similar effects on weight gain were seen in rats at 57 and 300 ppm of VDC. However, at 449 ppm of VDC, this effect on weight gain was not reversible. Feed consumption was reduced in rats at VDC concentrations of 57 ppm and above. Although feed consumption and weight gain were normal in mice exposed to 15 ppm of VDC, these parameters were reduced at higher concentrations.

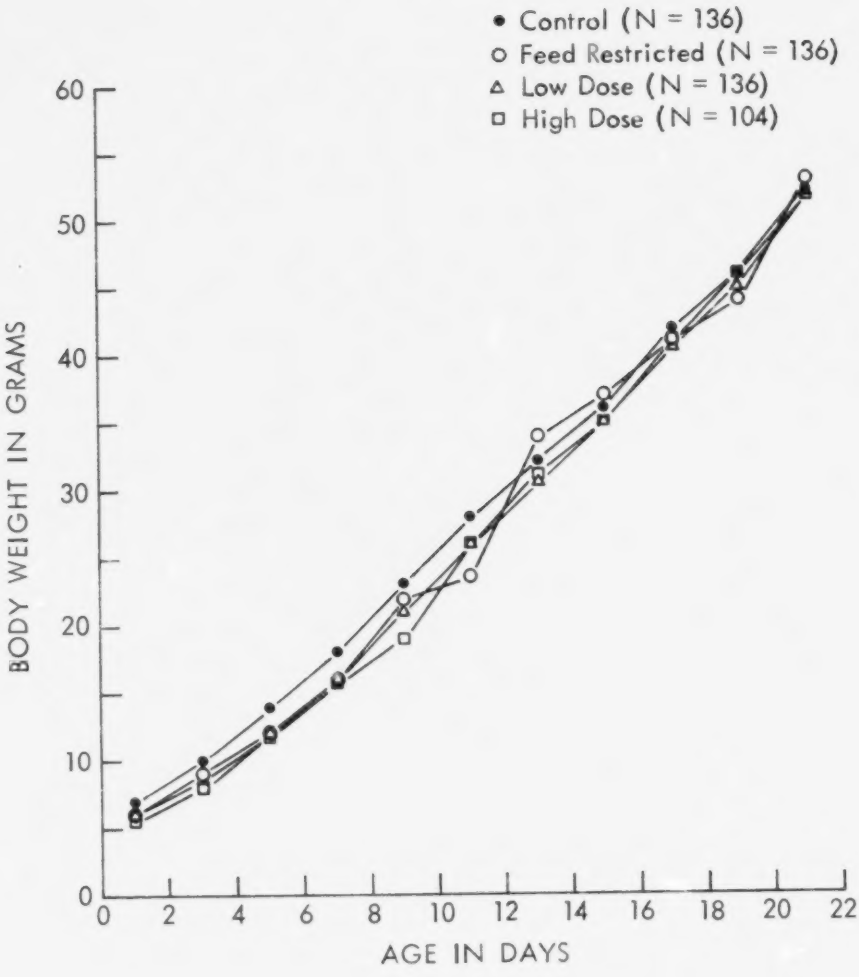

Figure 9 - Average Body Weight of All Rat Pups. All groups were significantly different from the control group ($p < 0.001$).

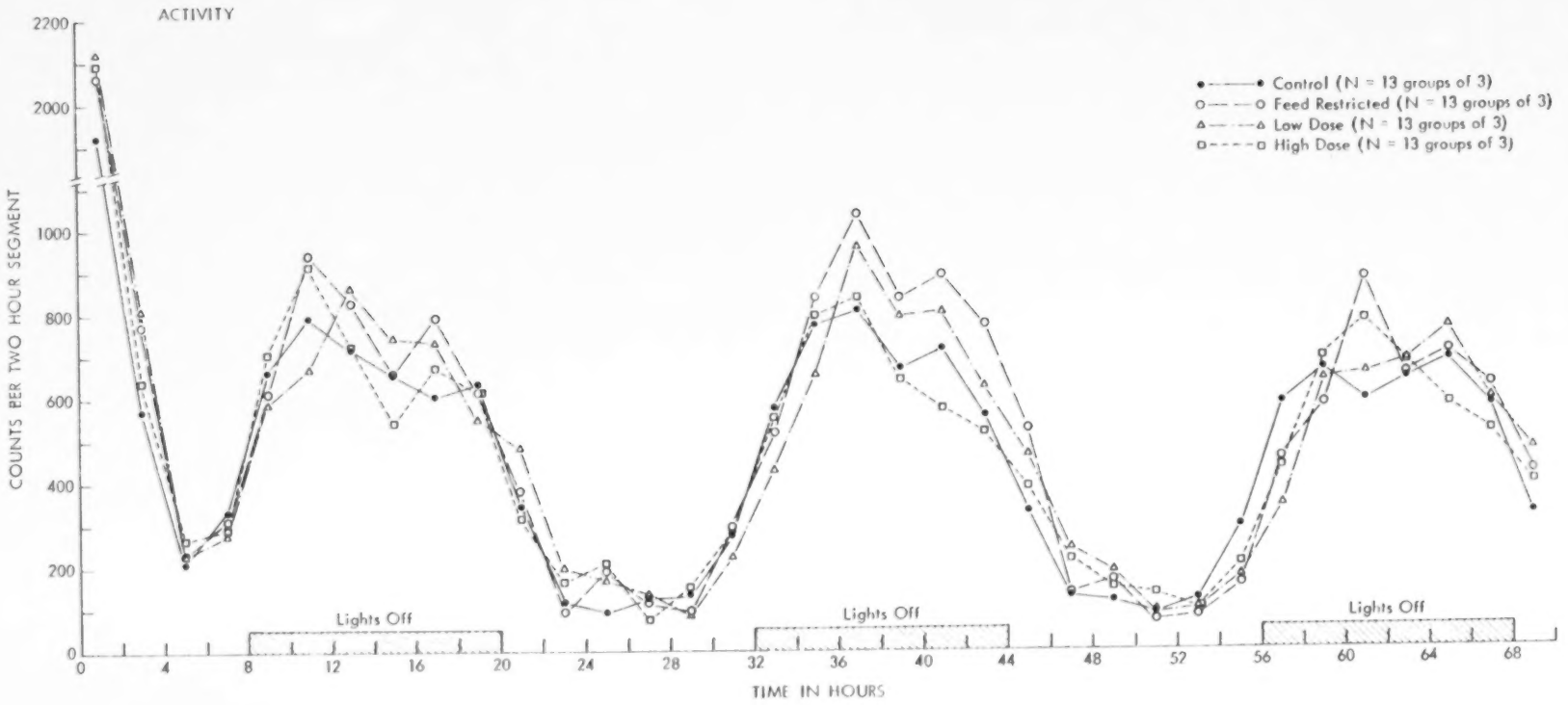

Figure 10 - Continuous Activity of Groups of Three Rats (same sex) in a Residental Maze, Both Sexes Combined.

Reduced feed consumption may be due either to toxicity of VDC in the adult or palatability problems associated with VDC contamination of the feed. Since the feed was changed daily in order to minimize possible contamination the former explanation is more likely. In contrast to these observations, virgin male and female mice survived a 6 hr/day, 5 day/week exposure to 55 ppm VDC without adverse effects on survival or weight gain (manuscript in preparation). These animals were given feed in the evening after the exposure period. These observations indicate that (1) the continuous exposure to low concentrations of VDC (15-41 ppm) is associated with adverse effects on maternal welfare which are generally reversible, (2) high concentrations of VDC produce both irreversible effects on maternal welfare and death, (3) mice are more sensitive than rats to the acute lethal effects of VDC, and (4) VDC is more toxic in mice during a continuous, rather than intermittent, exposure.

2. Reproduction and anomalies: Mice and rats were exposed to various concentrations of VDC from gestational days 6 to 16 in Part 1 of this study. This exposure was associated with a high incidence of early resorptions and an increased number of dams with complete resorptions in both mice and rats. These effects occurred in mice at 30 and 57 ppm of VDC (Table 2) and in rats at 57 and 449 ppm of VDC (Table 3). The surviving rat fetuses had a reduced body weight at VDC concentrations of 57 ppm and above. In addition, fetuses from dams exposed to 15 ppm of VDC and above had an increased incidence of hydrocephalus of the lateral ventricles (Table 5) and sternabrae anomalies (Table 7). No fetal mice were available for examination at VDC concentrations of 30 ppm and above, as a result of both maternal death and the high rate of resorptions. In this phase of the study, development in both rats and mice was altered at VDC concentrations that affected maternal welfare.

Since it is not possible to adequately evaluate the teratogenic potential of a compound with doses that produce a high incidence of resorptions, additional studies were designed. In these studies, VDC was administered for various phases of development. Such an exposure is valuable both in determining the sensitivity of the embryo to the compound at various stages of development and reducing the toxic effects of the compound. Mice were selected for these additional teratology studies because adult mice were more sensitive to the toxic effects of VDC than adult rats.

In Part 2, pregnant mice were exposed to 0, 41, 54 and 74 ppm of VDC for intervals that started at various times during gestation and ended on gestational day 15. This treatment was associated with a reduced weight gain during exposure at VDC concentrations of 41 ppm and above (Table 8). As a result of starting exposure on gestational days 8, 10 or 12 rather than 6, it was possible to reduce the incidence of resorptions and increase the number of viable fetuses (Table 9). However, exposures that started on gestational days 10 and 12 were still associated with a high incidence of resorptions. Consequently, exposures that started on gestational day 8 were

associated with fewer effects on development than exposures that started later even though all exposures ended at the same time. This observation suggests that (1) mice may adapt to a VDC environment after several days and (2) adverse effects on development, which were associated with VDC exposure, occurred later in development. According to this hypothesis, mice exposed to VDC from gestational days 8 to 15 adapted to the VDC environment during the first few days of exposure and, consequently, the embryos survived the critical period without any adverse effects. This hypothesis does not imply that VDC acts directly on the embryo to affect development. For example, the adaptation to VDC could be in terms of maternal physiology and the critical period could be that phase of development that is affected by such a change in maternal homostasis. VDC exposure was also associated with an increased incidence of both soft tissue and skeletal anomalies (Tables 12-15). However, this effect occurred at concentrations that adversely affected maternal welfare.

In Part 3, pregnant mice were exposed to 0, 56, 81 and 112 ppm of VDC for 2- to 3-day intervals during gestation. The feed restricted group, in this part, was starved during the exposure period in an effort to dissociate VDC related effects from those produced by malnourishment. All VDC concentrations were associated with a reduced weight gain of dams during at least one of the exposure periods (Tables 16-19). Starvation from gestational days 6 to 9 reduced the ratio of pregnant to plug-positive mice and increased the incidence of early resorptions (Table 16). Similar effects were seen in all the VDC exposed groups during this interval; however, the litters in the group exposed to 56 ppm of VDC appeared to be normal. Adverse effects on development were generally greater with VDC concentrations of 81 and 112 ppm than 56 ppm. The most severe pattern of anomalies seemed to occur when dams were exposed on gestational days 15 to 17. Hydrocephalus of the lateral ventricle and small kidneys occurred following VDC exposure (Table 24). However, since hydrocephalus occurred at a similar, although not statistically significant, rate in the feed restricted group, it is difficult to attribute this anomaly directly to VDC. There was, in addition, a diverse pattern of skeletal anomalies which occurred in both VDC exposed groups as well as the feed restricted group (Table 28). Ossification problems with the incus occurred more frequently in the VDC exposed groups; however, there was no dose related increase in this anomaly.

3. Summary: The morphological observations indicate that development in rats and mice is altered by exposure to VDC. However, these effects on development occur at VDC concentrations that adversely affect maternal welfare, as measured by weight gain, feed consumption, and survival. Since VDC interfered with development at doses that produced toxicity in the adult VDC was judged to have little primary effect on development.

B. Behavioral Study

1. Discussion: In Part 4, the behavioral development of rats, which were exposed *in utero* to VDC, was monitored. The results of this study tend to support the conclusion that the VDC treatment during pregnancy had no drastic effects on the subsequent behavior of the offspring. This conclusion then leads to the conclusion that VDC exposure of the dam during the period of gestation when brain development occurs, does not lead to neural impairment in the offspring.

On most of the behavioral tests there was no effect detected due to treatment. The high dose animals appeared to mature faster with respect to their ability to hang onto the horizontal bar, and the time at which the pinnae became detached. Also, along with the feed restricted group, the high dose group reached the first maturational level (Score 2) somewhat sooner in the swimming test. Since there were no differences at the other maturation levels, this effect on swimming is probably spurious.

Other significant differences were a slower maturation of the low and high dose groups for surface righting, the feed restricted and low dose groups for righting in air, and all groups showed later eruption of the incisors relative to the controls. One of the strongest effects was that on body weight. The feed restricted group was lighter than the controls, and both the low and high dose groups were lighter than the feed restricted group. These differences were gone by day 21.

There were no differences detected which were due to treatments in pivoting, auditory startle, eye opening, or adult activity levels. Of the significant differences discussed above, the slower maturation of the feed restricted and low dose groups in the righting in air test, and the marginal earlier maturation of the first level of swimming ability of the feed restricted and high dose group do not appear consistent with other data. They probably bear no practical significance.

In summary, the results indicate that the high dose rats matured at a faster rate with respect to bar holding ability and detachment of the pinnae. Both the feed restricted and treatment groups had depressed body weights, which may be related to the delayed eruption of the incisors observed in these groups. However, with respect to righting ability on a surface, the feed restricted group was successful the earliest and the low and high dose groups were significantly later.

2. Summary: No drastic problems with neural development were indicated. The VDC treatment may have altered the maturational rate of normal behavioral reflexes and patterns, with some maturing earlier, some later, and some not affected at all.

C. General Summary

The results of this study indicate that (1) VDC is more toxic in adult mice than adult rats, (2) adverse effects on maternal welfare, as measured by weight gain, feed consumption, and survival were observed in both mice and rats, (3) although morphological changes were observed in fetuses from dams exposed to VDC these effects were observed at concentrations that also affected maternal welfare, (4) no problems with neural development, as measured by behavioral parameters, were observed in rats exposed to VDC, and (5) VDC was judged to be only a weak teratogen with little primary effect on development.

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